AN *IN-VITRO* EVALUATION ON THE BIODEGRADABILITY OF RESILON

BY THE MICROBIOTA OF THE INFECTED ROOT CANAL

UTILIZING AN AGAR DISC DIFFUSION ASSAY

by

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INTRODUCTION

The primary goal of root canal treatment is the elimination of bacteria from the root canal system, which is the cause of pulpal and periapical infections.[1] After coronal access to the canal, rotary instruments, hand instruments, and chemical irrigation solutions clean, shape, and disinfect the canal. Subsequently, the canal space is obturated and sealed with a core material and a sealer. An adequate coronal restoration is then placed to seal the chamber where the access was made once obturation is complete.

Even after chemomechanical instrumentation, bacteria remain entombed within the dentinal tubules.[2] Therefore, it is ideal for an obturating material to be bactericidal or at least discourage bacterial growth. In addition, root canal treatment can fail as a result of microbial leakage after the obturating material has been placed.

Traditionally, gutta-percha has been used as an effective material to obturate the canals once cleaning and instrumentation are complete, although the material does not bond to the sealer or the dentinal walls. Also, gutta-percha is not impervious to microbial leakage. Gutta-percha is composed of 20-percent gutta-percha matrix, which is a coagulated latex (isomer of rubber), 66-percent zinc oxide as the filler, 11-percent barium sulfate as a radiopacifying agent, and 3.0-percent wax as a plasticizer.[3] Often obturation materials have been developed to prevent microbial leakage. Resilon is an obturation material introduced in recent years and claims to form a monoblock within the root canal system through the chemical bonding of the material to the dentin.

Resilon is a synthetic thermoplastic material that consists of a biodegradable polymer of polycaprolactone, bioactive glass as a filler, dimethacrylates, bismuth oxide, a

radiopacifying agent (barium sulfate) and coloring agents.[4] It is used with a dual-cured

methacrylate resin-based sealer and a self-etching primer in an attempt to bond the sealer

to the core material and the dentin walls resulting in a monoblock. This material has

generated great interest in the endodontic community and has been the subject of ongoing

research.

Recent research has shown that Resilon has some undesirable properties that

prompt questions about its use as an obturation material. First, the claim of a monoblock

has been brought into question. When this resin-based material is cured, polymerization

shrinkage occurs that can create gaps between the dentin and sealer where

microorganisms can penetrate and multiply.[5] Also, it is impossible to achieve complete

polymerization of the material especially in such long narrow spaces, and the

unpolymerized material at the dentin interface offers another leakage pathway for

bacteria. These unpolymerized monomers can leach out of the material into a wet

environment and promote bacterial growth. Others have questioned the stability of the

material itself. Tay et al. [6-7] have shown that Resilon is susceptible to alkaline and

enzymatic hydrolysis. Bacteria are able to release hydrolytic ester bond cleaving enzymes

that can act on the ester-linked methacrylate material within these polymer chains

composing polycaprolactone. The biodegradable material is ultimately converted to

water, carbon dioxide, methane (in anaerobic environments) and biomass. Resilon is

biodegradable by cholesterol esterase and pseudocholinesterase, which are both enzymes

present in saliva[8] and also by "dental sludge" or dental debris[9] collected by dental units.

Moreover, it is known that polycaprolactone degrades over time through a physical and

chemical process.[10] Polycaprolactone has been used in medicine for some time for its

biodegradable properties in absorbable sutures, subdermal contraceptive implants, and epidermal substrates for skin regeneration.[7] This has an aliphatic polyester component and is semi-crystalline in form. The crystallization of polycaprolactone affects its proven biodegradability because the poor adhesion between the polymeric matrix and ceramic particles results in early failure at the interface of the material and the dentin, which accelerates degradation of the composite's mechanical properties.[11] In addition, Melker[12] found that Resilon exhibits no antibacterial properties against bacteria that are found in the infected root canal system and periapical tissues such as *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Porphyromonas endodontalis, Actinomyces israeli, Actinomyces neaslundli,* and *Fusobacterium nucleatum.* The manufacturer and those that favor this material have criticized the degradation studies claiming that there is a discrepancy between bench work and clinical performance.[13]

Recently, the development of apical periodontitis has been noted in teeth obturated with Resilon in which there was no evidence of pre-operative apical periodontitis. Apical periodontitis is primarily an inflammatory disease of microbial etiology.[2]

In the early phase of root canal infection, facultative anaerobes fed by carbohydrates from the oral cavity will prevail. As infection progresses, obligate anaerobic bacteria dominate, because they can use tissue remnants and serum proteins as nutrients. "Endodontic treatment removes bacteria and necrotic debris from the root canal space, which leads to a drastic change in the root canal environment. When the root canal

is obturated, the root canal environment further changes, and gram-positive facultative or anaerobic microorganisms are often detected."[14]

Bacteria have virulence factors that allow survival in harsh environmental conditions like those present in persistent apical periodontitis, which is found in previously instrumented and medicated canals. When bacteria enter a starvation phase, they need an alternate carbon source and become extremely resistant to the irrigation solutions; therefore, resistant bacteria cannot be completely eradicated form the root canal system.

We have observed in clinical retreatment of teeth obturated with Resilon that the material shows color and consistency changes when the teeth have developed apical periodontitis. The material's color is no longer pink, but appears grayish-black and changes from the rigid state to a pliable, almost gelatinous form. Preliminary experiments have shown that standard Resilon cones incubated in bacterial media inoculated with human saliva undergo color change and darken (R.L. Gregory, personal observation), in a fashion similar to that seen in the root canals of retreatment cases where Resilon was used as the obturation material. The darkening appears to be related to the microbial composition of the saliva and not of the salivary proteins. This finding suggests that the microorganisms found in the oral cavity may be able to degrade Resilon. Therefore, the purpose of this study was to determine whether the microorganisms predominant in the infected root canal system have the capability to degrade Resilon.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

The first descriptions of dental disease and odontogenic pain date back to 14th century BC when the Chinese believed that the cause of tooth pain was due to a worm that created pain inside the tooth by eating the structure of the tooth.[15-16] Methods to treat the pain are found in the medical literature of the Romans, Greeks, and Chinese dating back to 1500 BC.[17] The oldest known root canal filling was a bronze wire within the pulp space of the lateral incisor of a Nabatean warrior alive around 200 BC.[18] In 1728 Pierre Fouchard refuted the tooth worm theory as the cause of odontogenic pain in the book *Surgical Dentist*. He gave accurate descriptions of the root canal systems and a technique by which the pulp chamber could be accessed to drain abscess or extirpate the pulps prior to filling the chambers with lead foil.[19] In 1757 Bourdet[20] described another way to treat diseased teeth by extracting and replanting them once the chambers were filled with gold or lead. In 1766 an American physician, Robert Woofendale, encouraged treatment of diseased pulp through cauterization within the chamber with a hot instrument.[19] In 1802 B.T. Longbotham was the first to recommend filling the roots of teeth deemed for extraction. However, Edward Hudson was the first clinician to perform this procedure by obturating the chamber and canal with gold foil.[19] Edwin Maynard was the person credited with developing the first root-canal instrument by filing a watch spring in 1838.[21] In 1847 Edwin Truman introduced gutta-percha as a filling material that consisted of gutta-percha, lime, powdered glass, feldspar, and metal.[22] In 1867 G.A. Bowman is credited as the first clinician to use gutta-percha cones as the sole obturating

material.[22] As these new materials, methods, and technologies were developed,

endodontic therapy began to gain acceptance in the early 1900s among dental

professionals. This growth was halted in 1910 when William Hunter, an English

physician, gave a lecture at the University of Montreal entitled, "The Role of Sepsis and

Antisepsis in Medicine," in which he accused dentists of "creating mausoleums of gold

over a mass of sepsis" that in turn lead to systemic diseases.[23] After this speech, the

practice and research in the field of endodontics largely ceased until the efforts of

courageous dentists such as C.N. Johnson, Callahan, Grove, and Howe, who began to

speak out against the extraction of all diseased teeth.[24] Finally, by the 1940s there was

sufficient laboratory and clinical evidence to confirm that devitalized teeth did not play a

role in the causation of systemic disease.[24] As the focal infection theory fell out of favor,

the advent of better radiography, bacterial culturing, diagnostic methods, and aseptic

techniques enabled the field of endodontics to gain acceptance.

In 1928 Harry B. Johnson coined the term endodontics and created the first

practice "limited to endodontics."[25] In 1943 a group of 20 dentists met in Chicago to

create an organization of endodontists.[26] The American Association of Endodontists was

formed with four goals: 1) To promote the interchange of ideas on methods of pulp

conservation and root canal treatment; 2) To stimulate research studies, both clinical and

laboratory, among its members; 3) To assist in establishing local root canal study clubs;

and 4) To help to maintain a high standard of root canal practice within the profession by

disseminating information through lectures, clinics, and publications.[27] In 1946 the first

Journal of Endodontics was published. The AAE continued to grow and the American

Board of Endodontics was established in 1956. Finally, in 1963 the American Dental

Association officially recognized endodontics as a specialty.

THEORY OF ENDODONTICS

The ultimate goal of endodontics is the elimination of the source of the infection

and the inflammation within the root canal system. In doing so, periapical pathosis is

eliminated and teeth are restored to a healthy state of proper form and function within the

masticatory apparatus.[28] A biological approach to endodontic therapy grounded in sound

research and clinical knowledge was advocated by Coolidge.[29] In a study of paramount

importance Kakehashi et al.[1] showed that pulpal pathosis did not occur in the absence of

bacteria. The pulps of germ-free rats were exposed and there was no development of

periapical pathosis at 42 days. Moller[30] demonstrated after inoculating sterile necrotized

exposed pulps with bacteria that noninfected 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. Due to bacterial

byproducts and toxins that initiate inflammatory mediators, periapical pathosis can also

be seen without the pulp being completely necrotic. A direct correlation exists between

the presence of a periapical radiolucency and the presence of bacteria, according to

Sundqvist.[31] To achieve endodontic success at every stage of RCT, the goal should be to

decrease or eliminate bacteria from within the root canal system.

The stages of RCT can be divided into chemomechanical preparation, microbial

control, and obturation as described by Stewart in 1955.[32] Each phase plays a key role in

healing of the periodontium, but Stewart along with many others believed that

chemomechanical preparation is the most important stage of therapy. Kuttler[33] was of the

opinion that proper obturation is the most critical step. He explains that ideal obturation thoroughly fills the canals and hermetically seals the canal system at the CDJ, which allows for the formation of a healthy periodontium with intact lamina dura. Schilder also believed that obturation is the most important phase of therapy and explained that when the root canal system is sealed from the periodontal ligament and the bone, this attachment apparatus could be protected from breakdown by endodontic pathogens.[34] Pitt Ford[35] also advocated the importance of ideal obturation because it: 1) diminishes space available to colonize bacteria; 2) prevents the contamination of the apex after pulp extirpation; and 3) prevents bacterial movement along the canal. Siskin[36] states that a canal without a hermetic seal will provide a means for tissue exudates to permeate and accumulate. These act as toxins and cause inflammation to the surrounding periapical tissues and will result in delayed healing or a perpetuation of periapical lesions and secondary infections. Healey[37] advocated that prior to initiation of RCT, the practitioner should consider the importance of case selection, accurate diagnosis, medical considerations, tooth essentiality, and operator ability. After obtaining the patient's consent and commencing RCT, 13 principles advocated by Grossman[38] should be followed as best practices:

1) Use aseptic technique.

2) Confine instruments to within the canal.

3) Avoid forcing instruments apically.

4) Enlarge the canal space from its original size to ensure proper debris removal.

5) Use copious amounts of antiseptic irrigation solutions throughout the procedure.

6) Retain irrigation solutions within the canal space.

7) Use only routine care for fistulas.

8) Obtain a negative culture before obturating the root canal system.

9) Create a hermetic seal at the cemento-dentinal junction.

10) Use obturation material that is not irritating to the periapical tissues.

11) Properly drain an acute alveolar abscess.

12) Avoid injecting into an infected area.

13) Use apical surgery when necessary to promote the healing of a pulpless tooth.

These principles are still considered today when evaluating any new endodontic
technique.

INSTRUMENTATION

Many endodontists believe that the cleaning and shaping of the root canal system
is the most important phase of treatment because proper chemomechanical
instrumentation facilitates elimination of the microbiota within the canal and allows for
ideal obturation.[28,39] The cleaning and shaping of canals includes removal of vital and
necrotic pulp tissue and infected dentin. In addition, this provides a pathway for the
irrigation solutions, medicaments, and obturation materials.[40] Today, there is a vast array
of instruments at one's disposal to help achieve the instrumentation of straightforward to
complex cases. The objectives during instrumentation are: 1) to create a continuously
tapering preparation that creates a resistant form and helps to prevent the overextension
of obturation materials; 2) To maintain the original anatomy of the root canal system and
refraining from removing excess dentin, which will weaken the tooth and make it more
subject to vertical root fractures; 3) To maintain the position of the apical foramen, 4)To

keep the apical foramen as small as possible; 5) To avoid iatrogenic damage to the root canal system and root structure that could negatively affect the prognosis; and 6) To avoid further irritation and infection of the periradicular tissues by forcing instruments with infected dentin outside of the apical constriction.[40-41]

Smith[42] compared different types of canal preparations in a study and the results at a five-year follow-up indicated that teeth prepared with more coronal flare were more successful (88 percent) when compared with those with a conical instrumentation technique (82 percent). This could be attributed to more effective cleaning and shaping of the middle and coronal thirds as well as easier access for instruments, irrigation solutions, and obturation materials.

IRRIGATION

Instrumentation must always be in conjunction with appropriate irrigation solutions in order to have the best chance at eliminating the infected dentinal debris and inflamed or necrotic tissue.[43] Harrison[44] states that irrigation solutions must possess these four properties to ensure endodontic success: 1) antimicrobial; 2) able to dissolve necrotic tissue; 3) helpful in the debridement of the canal system; and 4) biocompatible with sodium hypochlorite.

Sodium hypochlorite (NaOCl) is the most widely used endodontic solution in North America.[45] NaOCl is partially changed into hypochlorous acid (HOCl), which is capable of disrupting oxidative phosphorylation and DNA synthesis and thereby shows antimicrobial action.[46-47] When one considers the complexity of canal anatomy with lateral canals, accessory canals, isthmuses, fins, and the limitations of our endodontic instruments to operate in these areas, it is imperative to use irrigation solutions with

strong antimicrobial properties to reach infected dentinal tubules that are unreachable by instrumentation alone.[48] Another important quality of NaOCl is the property to break down the organic components of the root canal system.[49] This allows NaOCl to aide in the removal of necrotic and inflamed tissue as well as infected dentin chips. *E. faecalis* has been shown to be a very persistent and evasive bacterial species found within the infected root canal system due to its many virulence factors. When testing *in vitro* the antibacterial activity of NaOCl against *E. faecalis*, Gomes, and in a separate study, Radcliffe,[50-51] found that it took 5.25 percent concentration of NaOCl only 30 seconds to completely remove the microbe when in direct contact.

Chlorohexidine

Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that covers gram-positive and gram-negative bacteria. It is composed of a cationic molecular component that attaches and penetrates the negatively charged cell wall or outer membrane of microbes resulting in cell lysis and coagulation of intracellular components.[48,52] Chlorohexidine has been shown to have superior antimicrobial effect against *E. faecalis* when compared to NaOCl, killing the microbe in less than 30 seconds in concentrations of 0.2 percent or 2 percent.[50,53] In addition to its antimicrobial properties chlorohexidine has also been shown to have substantive properties for up to 12 days within the root canal system allowing for deeper penetration into dentinal tubules where persistent microbes might be housed.[54] Chlorohexidine is also well tolerated by the periapical tissues in contrast to NaOCl but does not possess the same property to dissolve the organic components of the root canal.

Ethylenediaminetetraacetic Acid

Ethylenediaminetetraacetic acid (EDTA) can effectively remove the smear layer by chelating the inorganic component of dentin.[55] Removal of the smear layer is an important step because this will allow for better penetration of antimicrobial irrigation solutions into previously blocked dentinal tubules in addition to the removal of the removal of dentin debris harboring bacteria, which is the main component of the smear layer.[56] A systematic review conducted by Shahravan[57] revealed that smear layer removal improves the fluid-tight seal of the root canal system, whereas other factors such as obturation technique or choice of sealer did not produce significant effects. This can also act as a lubricant for easier instrumentation of the canals with hand and rotary files.

OBTURATION

Once the root canal has been cleaned and shaped it is only then that satisfactory obturation is possible.[39] Root canal filling is indicated: 1) to help eliminate routes of leakage from the oral environment or periradicular tissues into the root canal space; 2) to prevent contamination of the periodontal tissues and subsequent periapical pathosis; and 3) to entomb any irritants that may remain in the root canal system.[58] The obturation of the root canal system is considered by some to be the most critical part of the endodontic treatment.[59] Coolidge[29] states that the primary purpose of root canal obturation is to seal the apical foramen as well as obliterate the canal.

As practitioners we have always had many options for materials and techniques as to how we prefer to obturate the cleaned and shaped root canal system. Glickman and Gutmann[60] said the practitioner must recognize "no one particular obturation technique will satisfy the myriad of clinical cases that require endodontic therapy." There have

been a variety of obturation materials used over the years ranging from asbestos to bamboo.[18] Prior to the 19th century, root canal fillings were limited to gold and other metals such as zinc and amalgam. By definition, a root canal filling material is any material or combination of materials placed inside a root canal system for the purpose of obturating and sealing the canal space. This usually consists of a sealer or a cement and a solid or a semi-solid core material.[61] Grossman[18] provides a list of attributes the ideal root canal obturation material should possess:

1) Easily introduced into the root canal system.

2) Liquid or semisolid state that becomes solid.

3) Able to seal apically and laterally.

4) Able to withstand shrinkage.

5) Impermeable to moisture.

6) Bacteriostatic.

7) Non-staining.

8) Unthreatening to the periapical tissues.

9) Easily removed.

10) Sterile or sterilizable.

11) Radiopaque.

Silver Points

In the late 1920s silver cones were introduced as a root canal filling material. They were considered inert, stable, and nonresorbable. Due to their rigidity, they could be wedged into the critical apical third and provide a hermetic seal when used in conjunction with sealer. Generally, oversized cones were shaved down into a gentle taper that

permitted the silver cone to be locked into position and unable to be forced deeper or removed without considerable effort.[34] However, most canals are not circular in nature, and thus silver cones did not fill canals in their entirety. The combination of the lack of an adequate seal three-dimensionally due to the stiffness of the point and the propensity for corrosion[62] over time has made this technique below the standard of care.[63]

Chloropercha

In 1974 the chloropercha technique was described by Bence.[64] Using this technique, the gutta-percha is dissolved into chloroform until a syrup-like consistency is achieved. Once the canal is prepared with a definite apical stop, the master gutta-percha cone is dipped in the chloropercha, which acts as a sealer, and the cone is then inserted to working length. Accessory points are added in normal fashion until a dense obturation is achieved. When using chloropercha and vertical compaction, the master point is trimmed to 4 mm to 5 mm and attached to a warm plugger. This is then dipped into chloropercha, seated to working length, and condensed. This is repeated with subsequent portions of gutta-percha dipped in chloropercha until the canal is completely obturated. The chloropercha technique has fallen out of favor due to problems with evaporation of the solvent and with shrinkage of the material after evaporation, which caused a poor apical seal and greater leakage.[65-68]

Paste

There have been several paste-type obturation materials developed as an alternative to gutta-percha due to the potential for excellent canal adaptation. However, in research examining PCA, ZOE, Endo Fill and Hydron paste-filling materials, while they

exhibited less dye penetration than gutta-percha and Roth sealer, they showed microscopically to result in voids throughout the fill.[69]

The most popular paste system was introduced in 1950 by Angelo Sargenti and was known as N2 or Sargenti's paste. It contained a mixture of hydrocortisone, two antibiotics, paraformaldehyde, and zinc-oxide eugenol.[70] This method lost support for its use after reports that the material was highly inflammatory and cytotoxic. Newton[71] conducted research in 1980 that showed inflammation and osteomyelitis present in the jaws of monkeys at 6 months and 1 year following treatment with N2 paste. There have also been reports of permanent paresthesia when overextension of N2 paste was in close proximity to the inferior alveolar nerve.[72]

Cold Lateral

Lateral condensation can be performed with a conservative or larger preparation and minimizes the risk of overextension of the material into the periapical tissues. Other advantages of this technique include relative ease of use, low cost, and predictability. Glickman and Gutmann[60] list several important conditions that must be met when performing lateral condensation. First, the shape of the prepared root canal must be continuous to allow for the proper placement of the master cone, spreader, and accessory cones. Second, the forces in lateral condensation are both vertical and lateral. Third, the placement of the spreader must reach the appropriate depth without touching the canal walls to ensure continuous taper prior to condensation. Fourth, the master cone should fit within 0.5 mm to 1.0 mm of the radiographic apex and should have tug back. Fifth and lastly, the placement of the spreader should be within 1 mm of the working length adjacent to the master cone.

The technique consists of coating the master cone with sealer and placing it to working length. Next, the appropriate spreader is selected and carefully removed with a watch-winding motion without pulling the master cone out. This space allows room for a sealer coated accessory cone. This process is repeated until the spreader advances no further than 2 mm to 3 mm from the canal orifice. Heat is applied to remove the excess material, and this is followed by apical compaction.

According to results in several studies, lateral condensation has been shown to provide a better apical seal than the single-cone technique, Thermafil, or Soft-Core.[73-74] One of the drawbacks to this technique, in addition to being time-consuming, is the possibility of iatrogenic vertical root fracture during compaction due to the forces applied during condensation. Meister[75] examined 32 cases of vertical root fracture and found that the cause of the fracture was lateral condensation in 27 (84 percent) of the cases. In addition to the increased chance for vertical root fracture, the final obturation is comprised of several gutta-percha cones coupled by frictional grip and sealing material, rather than a homogeneous mass of gutta-percha.[76] Voids can be easily created through poor root canal preparation, inadequate lateral pressure, curved canals, mismatch between spreader size, and accessory cone, all resulting in a heavy reliance on the sealer to fill the voids, implicating a poorer prognosis than if a homogeneous mass were used.

Warm Vertical

Vertical condensation was first introduced by Schilder[34] in 1967 as a way to obturate the canal in three dimensions by reaching all ramifications of the root canal system rather than just the main canal. He states that this method offers advantages of

dimensional stability, high density in the apical portion of the canal, and the ability of the

obturation material to flow into lateral and accessory canals.

Schilder's technique consists of selecting a master gutta-percha cone that closely

approximates the canal shape, applying sealer to the cone, and seating to within 1 mm to

2 mm of the apical extent of the preparation. Heated pluggers remove the coronal aspect

of the material and transfer heat to the remaining apical portion of the cone, and it is

condensed vertically and laterally. The remaining portion is obturated by placing heated

gutta-percha segments until the coronal portion of the canal is filled.

Glickman and Gutmann[60] offer some guidelines to be considered when using this

technique: 1) The canal must have a gradual taper; 2) The internal cross-section of the

canal should continuously get wider from the prepared apical constriction; 3) No apical

zipping, perforation, or blocking can be present; 4) The master cone must mimic the

shape of the prepared canal; 5) Only a small amount of sealer should be used in order to

reduce the expression of sealer out of the apical foramen; 6) Gutta-percha must be

softened to allow for apical movement of the material; 7) Apical condensers should never

bind in the canal walls preventing apical penetration of the instrument.

Brothman[77] showed that warm vertical condensation demonstrates more lateral

canals (34 percent) when compared with cold lateral condensation (6 percent). Research

by Nelson et al. and Lea[78-79] found that when heat was applied through electronically

heated pluggers such as System B there was a higher density (27 percent increase) of

gutta-percha in the apical third when compared with lateral condensation. Aqrabawi[80]

assessed success rates of lateral vs. warm vertical condensation using clinical and

radiographic signs and the results showed a significantly higher success rate for the teeth

obturated with warm vertical obturation. However, in a meta-analysis conducted by Peng[81] comparing warm vertical obturation to cold lateral, there were similar outcomes seen between the two groups in regards to postoperative pain, long-term outcomes, and obturation quality. Warm vertical obturation demonstrated a higher rate of overextension than cold lateral.

Continuous Wave

Buchanan[82] modified the warm vertical technique by introducing the System B heat source, which delivers continuous heat for extended periods of time known as continuous wave obturation. With this technique after placement of the master cone, the System B is activated, and heat is delivered to the preselected plugger at the recommended temperature setting. The plugger is advanced through the gutta-percha until it is 5 mm to 7 mm from the apical extent of the canal. Apical pressure is held for 10 seconds and then additional heat is applied for one second. Then, the coronal portion of the master cone is removed. The remaining portion of the canal can be obturated using an injectable system of gutta-percha, introduced by Yee,[83] that delivers thermoplasticized gutta-percha to the canal and then condenses with cold pluggers.

Pommel[84] investigated the apical leakage of continuous wave and found it to be as effective as warm vertical condensation and Thermafil and superior to that of cold lateral. Venturi[85] evaluated the quality of apical seal in narrow and curved canals using four variations of warm vertical condensation through the use of a dye-penetration model, and the results showed vertical condensation as well as continuous wave were very effective at sealing the canals and provided excellent adaptation of the materials to the root canal walls.

Carrier-Based Obturation

Carrier-based obturation is a technique that has gained favor among general dentists due to ease of use. With this system a carrier (usually plastic-based) is coated with gutta-percha and warmed in a tailor-made oven until material is of a consistency allowing it to flow into the instrumented root canal. Prior to its placement, the canal is coated with the sealer of choice. While a benefit is its ease of use and time efficiency, some reported drawbacks are the stripping of the obturation material off the carrier before it reaches the desired working length, possibly compromising the apical seal. In addition, because it is easily forced into various size preps, there can be a tendency to underprepare canal systems, and if these cases fail and retreatment is necessary, carriers are historically challenging to remove. The latter is because they are found bound tightly into canals and up against walls.

In regard to clinical success, Chu[86] found no difference in a 3.5 year follow-up between teeth obturated using Thermafil, a popular carrier-based obturation system, and those obturated with lateral condensation of gutta-percha. It has been shown by DuLac[87] that carrier-based obturation can fill lateral canals as well as the continuous wave technique and better than warm vertical or lateral techniques. In terms of sealability, Thermfil has been shown to have significantly less leakage and more core material present within the canal, in a dye leakage study in which carrier-based obturation systems were compared with lateral condensation.[88] However, in a study by Gutmann[89] it was shown there was no statistical difference between lateral condensation and Thermafil and that both were considered good obturation techniques in terms of sealability.

Recently, a new carrier-based product was introduced, GuttaCore, which is composed of a crosslinked gutta-percha carrier in place of the more popular plastic ones. This new product was introduced in hopes of addressing some drawbacks of Thermafil. GuttaCore hopes to eliminate the problems faced with retreatment of the material, because the core is physically the same as gutta-percha with the exception of its resistance to melting from heat. In addition, the material cannot be forced into underprepared areas, because Thermafil could will not glide into place, but crumbles or breaks. Therefore, operators using this technique will have to prepare the canals to larger apical diameters, and this preparation will clean them more effectively.

Gutta-Percha

In 1847 gutta-percha root canal filling material was developed by Hill, and it was first known as "Hill's stopping," consisting primarily of gutta-percha, lime, and quartz.[90] Gutta-percha today is currently the most widely used core obturation material.[58] Gutta-percha formulations can vary among manufacturers but usually contain 56 percent to 80 percent zinc oxide with antimicrobial properties,[91] 19 percent to 30 percent gutta-percha, 1.0 percent to 17 percent heavy metal sulfates for radiopacity, 1.0 percent to 4.0-percent waxes and resins for compactibility, and 0.3-percent to 1.0-percent pigments.[92] Pure gutta-percha can be found in alpha or beta crystalline forms. Most commercial gutta-percha exists as the beta form, which is more soft and pliable. The alpha form is the natural form from the Taban or Isonandra percha tree.[92] The alpha form is a 1,4-polyisoprene, which is harder, more brittle, and less elastic than natural rubber.[92] Schilder[93] demonstrated that the two forms are affected by changes in temperature. When heated from root temperature to 42°C to 49°C, a beta to alpha transformation takes

place, and then alpha changes to an amorphous state between 53°C to 59°C. During

cooling, gutta-percha transforms back to the beta phase and can undergo significant

shrinkage; therefore, continuous compaction is necessary during cooling. The material is

considered to have acceptable biocompatibility and a low degree of toxicity.[94] When

Tavares et al.[95] implanted gutta-percha into rats, it proved to be well tolerated by the

connective tissues. It has been shown by Sjogren[96] that when gutta-percha is extended

beyond the radiographic apex of the tooth, there is a significantly lower rate of success.

RESILON

Gutta-percha has been the obturation material of choice for many years. Used

with great success, it is considered the gold standard because it possesses many features

of an ideal root-filling material. However, it does not bond to tooth structure and its

ability to prevent leakage has been questioned.[97-98] Other reported drawbacks are the

inability to completely remove it during retreatment[99] and that it does not strengthen

endodontically treated teeth.[100] Resilon, a resin-based obturation system has been

developed as an alternative to gutta-percha in an attempt to improve upon these

drawbacks by forming an adhesive bond or monoblock between the core material and the

sealer as well as the dentin and sealer.

Introduced in 2004, Resilon is a thermoplastic, synthetic, polymer-based

obturation material used with a self-etching resin sealer RealSeal SE. Resilon is

composed of dimethacrylate resin, bioactive glass as a filler, bismuth oxychloride,

radiopaque barium sulfate, and polycaprolactone.[4] Polycaprolactone is a polymer of

polyester used in medicine for some time for its biodegradable properties in absorbable

sutures, subdermal contraceptive implants, and epidermal substrates for skin

regeneration.[7] This has an aliphatic polyester component and is semi-crystalline in form. The crystallization of polycaprolactone affects its proven biodegradability because the poor adhesion between the polymeric matrix and ceramic particles results in early failure at the interface of the material and the dentin that accelerates degradation of the composite's mechanical properties. When Resilon is used in conjunction with RealSeal sealer, the obturation system should be impenetrable by microorganisms[101] due to the formation of a monoblock within the canal.[102]

In an *in-vitro* experiment testing the likelihood of Resilon to form a "hermetic seal," Tay[103] observed gaps between the sealer dentin interface in the Resilon/Epiphany group and gaps between the gutta-percha and AH26, leaving the sealer on the dentin surface and within the dentin tubules in the gutta-percha group when viewed by SEM. The authors concluded that a "hermetic seal" was not achievable with the use of either obturating material. These gaps were likely due to the fact that when this resin-based material is cured, polymerization shrinkage occurs, which can create gaps between the dentin and sealer where microorganisms can penetrate and multiply.[5] Also, it is impossible to achieve complete polymerization of the material, especially in such long narrow spaces, and when the unpolymerized material at the dentin interface offers another leakage pathway for bacteria. These unpolymerized monomers can leach out of the material into a wet environment and promote bacterial growth. In addition, several studies indicate that Resilon exhibits no antibacterial properties[12, 104-107] against bacteria that predominate the infected root canal system and periapical tissues such as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida*

albicans, Porphyromonas endodontalis, Actinomyces israeli, Actinomyces neaslundli,
and *Fusobacterium nucleatum.*

Application

The technique for using Resilon is similar to that of gutta-percha. The cone or the
canal walls are coated with sealer after complete instrumentation and irrigation. Once the
Resilon is used for obturation, regardless of method used, the manufacturer claims that an
immediate coronal seal will be produced by light curing for 40 seconds, while the
remaining sealer will set in 25 minutes. However, in an aerobic environment, it has been
found that the setting of the material can take up to three weeks. This suggests that
Resilon will not completely set in the periradicular tissues if it were to be extruded.[108]

Clinical Findings

In a direct comparison between Resilon and gutta-percha, there was found to be
no significant difference between the clinical outcome of both materials at one-year and
two- year follow-ups.[109] Another study showed similar results of healing rates among
Resilon and gutta-percha-treated teeth when comparing one-year post-treatment
radiographs.[110] However, the review period in both studies was short; the numbers of
teeth examined was relatively small; there was no clinical examination, and the
investigators had a financial interest in the product being investigated in one study.

Recently, the development of apical periodontitis has been noted in teeth
obturated with Resilon when there was no evidence of pre-operative apical periodontitis.
We have observed in clinical retreatment that teeth initially obturated with Resilon and
that subsequently developed apical periodontitis have shown color and consistency

changes with the material. The Resilon was no longer a pinkish color but appeared grayish-black and had changed from the rigid state to a pliable, almost gelatinous form.

Previous Research

Tay and Pashley have heavily researched several aspects of Resilon in an attempt to answer questions such as whether a monoblock can be achieved, and if the microorganisms have access to the core Resilon material, whether it can be degraded. They have shown that Resilon is susceptible to alkaline and enzymatic hydrolysis.[6-7] Bacteria are able to release hydrolytic ester bond-cleaving enzymes that can act on the ester-linked methacrylate material within these polymer chains. The biodegradable material is ultimately converted to water, carbon dioxide, methane (in anaerobic environments) and biomass. In addition, Resilon was shown to be biodegradable by cholesterol esterase and lipase, which are both enzymes present in saliva or can also be secreted by bacteria found in infected root canal systems. Resilon exhibited significant surface thinning and weight loss after incubation with these enzymes[8] and also by "dental sludge" or dental debris collected by dental units.[9] The manufacturer and those that favor this material have been critical of these works claiming that they have been done *in vitro*, and that there is a discrepancy between bench work and clinical performance.

Sealers

Gutta-percha does not bind to root canal walls; a sealing agent must be used to obtain a "hydraulic closure" of the canal system by filling the irregularities of the canal.[111] The ideal sealer should possess the following properties:

1) Non-irritating to the tooth and periapical tissues.

2) Hermetic sealing ability.

3) Bactericidal or bacteriostatic activity.

4) Non-staining to the tooth.

5) Insoluble in tissue fluids.

6) Radiopaque.

7) Exhibits good adhesion to dentin.

8) Long working and setting time.

9) No setting shrinkage.

10) Good mixing consistency.

11) Soluble in a common solvent.

ZOE-based sealers such as Roth or Grossman's have been considered the sealer of choice due to the antibacterial properties, ease of use, and sealing properties.[112] Leonardo[113] used an agar diffusion method that showed zinc oxide exhibiting antimicrobial properties against both gram-positive and negative bacteria. Pupo[114] tested the antimicrobial properties of zinc oxide-containing cements and found that they inhibited growth of both gram-positive and negative bacteria as well.

RealSeal SE, formerly known as Epiphany, is a dual-cured resin composite self-etching sealer, which allows auto-polymerization within the canals.[103] The resin component of the sealer comprises 30 percent and is made up of BisGMA, ethoxylated BisGMA, UDMA, and hydrophilic difunctional methacrylates. The remaining 70 percent is composed of fillers: calcium hydroxide, barium sulfate, barium glass, and silica.[115] When this sealer is used in conjunction with Resilon core material, manufacturers claim

the sealer bonds to the dentinal wall and the core material to form an obturation that should prevent leakage.

In an *in-vitro* experiment comparing the microbial leakage between gutta-percha with AH26 and Resilon with Epiphany, the former had 73 percent to 93 percent of samples exhibiting crown-down leakage while only 7 percent to 13 percent of the Resilon samples demonstrated contamination at the apex.[115] Nazzal, in her graduate thesis, has shown that when comparing the sealability of Roth's sealer to Brasselers's Bioceramic Sealer, Roth's provided a better seal against microleakage. Several problems with the Epiphany sealer used in this monoblock system have been encountered. Baumgartner[116] found Resilon/Epiphany exhibited more leakage against *E. faecalis* when compared with gutta-percha/AH Plus. In a similar study, it was found that Resilon/Epiphany root fillings initially prevented fluid movement to the same degree as gutta-percha/AH Plus counterparts, but when tested at 16 months, 29 of the 40 specimens exhibited gross leakage similar to positive controls.[117] It has been shown that the seal of the resin-based sealer Epiphany to Resilon decreases substantially over time and that an epoxy-based sealer Ah-26 actually has a stronger bond to Resilon.[115] In addition to the increased microleakage exhibited by Epiphany, Slutzky-Goldberg et al.[118] were able to show that Epiphany SE actually enhanced bacterial growth of *E. faecalis* when compared with other traditional sealers. In addition, the self-etch/self-adhesive sealers must be able to etch beyond the smear layer in order to reach dentin to achieve micromechanical retention. Kim[119] showed that the newer fourth-generation sealers due to the higher pH are not capable of this. Even if a hermetic seal was achievable and if the bond of the sealer to dentin did achieve a monoblock, it is unlikely that 100 percent of the Resilon cone is

coated with sealer once in place; if coronal leakage were to occur, these sealers are biodegradable by cholesterol esterase and pseudocholinesterase, which are both enzymes present in saliva.[6]

SUCCESS RATES OF ENDODONTIC THERAPY

Knowledge of the success and failure of root canal therapy shown in outcome studies is paramount to proper clinical decision making and being able to inform your patients of achievable results. A key to analyzing outcome studies is the definition of success, which in most endodontic literature can be defined as relief of symptoms, tooth presence and functionality, and a resolution of periapical radiolucencies or a reduction in size considered an apical scar. Many factors should be evaluated that weigh heavily on the prognosis of treatments such as initial vs. retreatment, presence or absence of periapical radiolucency, single or multi-rooted canal, and single versus two-visit therapy.

Bystrom[120] has shown that when a tooth is only mechanically instrumented the bacterial load is decreased by 20 percent to 43 percent. However, when chemomechanical instrumentation is accomplished, this reduction is greater at 40 percent to 60 percent for the microorganisms present. Finally, when a tooth is chemomechanically instrumented in addition to placement of $Ca(OH)_2$ the reduction in bacteria reaches 90 percent to 100%. From this, we can gather the importance of chemicals such as NaOH and $Ca(OH)_2$ in the overall reduction of bacterial load and cannot rely on physical instrumentation of our files alone. However, in a systematic review conducted by Sathorn,[121] it was found that $Ca(OH)_2$ has limited effectiveness in eliminating bacteria from the root canal when assessed by current culture techniques. With this in mind, many authors have found no statistical difference between one and two-visit endodontic therapy treatment. Oliet[122]

stated he found no increase in post-operative pain or healing when treatment was performed in one or two visits at an 18-month follow-up. Pekruhn[123] also found one-step RCT to be successful 94.8 percent of the time, but he did note an increase in flare-up percentage when there were periapical radiolucencies (PARL) present or in cases of retreatment. In a meta-analysis Sathorn[124] found no statistically significant difference between one and two-visit RCT and actually showed one visit to have a 6.3 percent higher healing rate. The same conclusion was found in systematic reviews by Figini[125] and Su[126] stating there is no statistical difference in radiologic success when comparing single-visit treatment with multiple visits. Su[126] also found the prevalence of post-obturation pain to be significantly lower in single visit vs. multiple visit groups. Eleazor[127] found there to be an 8-percent chance of flare-up if RCT was performed in two visits vs. 3 percent of cases resulting in flare-ups if treated in one visit. In summary, the literature does not support or refute one-visit RCT over two-visit RCT and practitioners have seen high success rates with either mode of treatment.

The importance of a good coronal restoration is paramount when trying to achieve the highest success rate for RCT. A classic article by Ray[128] radiographically examined 1010 endodontically treated teeth restored with a permanent restoration and found the quality of the coronal restoration was significantly more important than the quality of the endodontic treatment for the presence of apical periodontitis. In a recent systematic review by Gillen, teeth with apical periodontitis have a better prognosis with both an adequate RCT and restorative treatment.[129] Another systematic review by Stavropoulou[130] found that the 10-year survival rate for crowned RCT teeth is 81 percent

while it drops to 63 percent when teeth have only direct restorations such as amalgams and composites.

Epidemiological studies have found prognostic indicators that contribute to the success rate of root therapy. Lazarski[131] found many factors that negatively contributed to the success rate of endodontically treated teeth: no coronal coverage, intraoperative complications (file separation or perforation), and history of a flare-up. Nevertheless, after looking at ~45,000 teeth with a 3.5-year follow-up, the success rate was still 94.4 percent. Salehrabi[132] found a similar success rate of 97 percent when RCT was performed by general dentists and endodontists alike with various pre-op diagnoses. In this study 1.46 million teeth were found to be retained eight years after RCT. Another important finding from this was that of the 3-percent failures, the majority occurred within three years. Of these failures, 85 percent never had any full coronal coverage placed. In research conducted by Alley,[133] it was found that despite a larger number of difficult cases treated by endodontists, their success rate of 98.1 percent was only slighter greater than that of general practitioners at 89.7 percent.

Imura[134] compared success rates found in primary vs. retreatment RCT in ~2000 teeth and found an overall success rate of 91.45 percent. The success was slightly higher, 94 percent among initial treatments when compared with retreatments at 86 percent. Sjogren's[135] research provided even more insight into predictive factors by separating initial and secondary treatment and those with and without periapical radiolucencies among 471 teeth. He found that initial RCT without AP had a 96-percent success rate, but if a lesion was present, the rate dropped to 86 percent. In cases of retreatment without AP, there was a success rate of 89 percent, but only 62 percent if the case presented with

a PARL. In a recent systematic review evaluating the effectiveness of primary root canal treatment, it was found that the outcome of primary treatment improved significantly in the absence of a PARL, and in treatments using obturation without voids, oburation extending to 0 mm to 2 mm from the radiographic apex, and a satisfactory coronal restoration.[136]

Failure of the root canal treatment can result from a number of reasons, but leakage of fluid, microorganisms, or microbial by-products through the root canal filling material is thought to be a major contributing factor. In a meta-analysis determining the optimal obturation length, it was shown that when obturated 0 mm to 1mm from the apex, the best results were seen, although not statistically significantly different from results obtained at greater than 1 mm. Both groups showed statistically significantly better healing when compared with those obturated past the apex.[137] It was also shown by Sjogren[96] that when gutta-percha is extended beyond the radiographic apex of the tooth, there was a significantly lower rate of success. Similarly, Seltzer et al.[138] found that overfilled root canals were significantly less successful than those obturated flush with the apex or slightly underfilled. Furthermore, it has been reported that when there was a lesion present and initial RCT was performed within 0 mm to 2mm of the radiographic apex, the success rate was 94 percent. This dropped to 76 percent if cases were obturated long and to 68 percent if cases were obturated more than 2 mm away from the radiographic apex.[96]

The Toronto study looked at ~550 teeth and found that those without apical periodontitis (AP) healed 93 percent of the time compared with 82 percent of the time when AP was present prior to RCT. The overall success rate was 86 percent.[139] This

study confirmed apical periodontitis as the main prognostic factor in initial endodontic treatment. Other variables that had a significant effect on the outcome were the length of the obturation (87 percent healed when adequate and only 77 percent if the length was inadequate), single or multi-rooted (93-percent success if single root vs. 84 percent in multi-rooted teeth), lateral condensation showed healing less than warm vertical (77 percent vs. 87 percent), and if there were no intraoperative complications the success rate was higher (84 percent) as compared with when these complications occurred reducing the success rate to 69 percent.[139]

Various non-surgical and surgical treatment options and likely outcomes can be explained to the patient, in view of data from recent studies regarding the success rates of endodontic surgery. Testori[140] found that with improvements made in the microsurgical field of endodontics over the last decade, these have significantly affected the success rates of surgical RCT. In a meta-analysis by Setzer,[141] it was shown that when comparing traditional root-end surgical materials (TRS) and techniques to the more recent endodontic microsurgery (EMS), the probability for success was 1.58 times greater in the EMS group showing a 94-percent positive outcome compared with 59 percent in the TRS group. When performing ultrasonic preparation and retrograde filling with super-EBA by using a surgical operating microscope, the success rate at 4.5 years follow-up was 86 percent compared with a previously reported success rate of 68 percent when using rotary handpieces with round burs and amalgam. Rubinstein[142] found similar results when using ultrasonics, superEBA, and a microscope to perform surgical RCT with a success rate of 91.5 percent at a five-year follow-up. Von Arx[143] reviewed a meta-analysis of prognostic factors in apical surgery outcomes that concluded that teeth

without preoperative pain, dense root canal filling, and 0 mm to 5mm apical lesions had higher healing rates than those with pain, poor obturation, or large lesions. These studies depict the benefits of endodontic therapy with a high likelihood of achieving a favorable outcome when a good periodontal and restorative prognosis is anticipated and the patient is motivated to save their tooth.

MICROORGANISMS OF THE ROOT CANAL

The primary goal of RCT is to rid the root canal system of as many microorganisms as possible. Bacteria must be present to cause infection and periapical radiolucencies[1, 30] and microorganisms can enter the root canal system by way of caries,[144-145] fractures,[146-147] exposed dentinal tubules,[148] coronal leakage,[149-150] via the peridontium,[151] and possibly through anachoresis.[152-153] Once these microorganisms enter the pulp, they must be able to evade host defense mechanisms, initiate tissue destruction, colonize, and survive in the host environment. Much of this can be accomplished by the virulence factors possessed by the particular bacterial species.

There are many virulence factors that bacteria possess which enable them to successfully colonize within the host. Endotoxin (LPS) can cause bone resorption and exaggerate inflammation.[154-155] Various enzymes are capable of collagen destruction, digestion of ground substance, proteolytic activities, and destroying host cells.[156-158] The metabolic byproducts of bacteria have the capability to destroy host cells and protein.[159] Some possess collagen binding proteins that allow them to adhere within the dentinal tubules more effectively.[160] *E. faecalis* has a proton pump that it to survive $Ca(OH)_2$ treatment.[161] In a systematic review by Estrela,[162] it was concluded that NaOCl and CHX both showed low antimicrobial efficacy to eliminate *E. faecalis* during root canal

disinfection. Through quorum sensing they are able to communicate and coaggregate with each other.[163-165] Some bacteria possess fimbriae that contribute to their pathogenicity.[166-167]

The species seen most often in primary endodontic infections are predominantly anaerobic gram negative rods such as porphyromonas, prevotella, fusobacterium, and bacteroides.[168] Candida can be seen as well in primary infections.[169] Most primary infections are polymicrobial with molecular studies showing anywhere from 1 specie to 17 species (4.7 mean) within the root canal system and 1 specie to 33 species (5.9 mean) if it presents with a periapical abscess.[170-171] The apical areas are dominated by slow-growing obligate anaerobes while the more coronal portions house rapidly growing facultative anaerobes.[172] In previously treated canals, a mixed flora is seen, but gram-positive anaerobic cocci are often found, especially *E. faecalis*.[173] In secondary infections the molecular studies still show multiple species but usually this number will be lower than primary invaders. Microorganisms seen in periapical lesions are polymicrobial and predominately gram-positive anaerobes as well as fungi and viruses. Actinomyces is seen frequently in secondary infections.[174]

The bacteria tested in our research represented a wide variety of microorganisms seen in the infected root canal system. The tested bacteria fell into the following catagories: facultative gram-positive cocci (streptococcus, staphylococcus, and enterococcus), obligate gram-positive cocci (peptostreptococcus), obligate gram-positive rods (actinomyces), facultative gram-negative rods (pseudomonas), obligate gram-negative rods (porphyromonas, prevotella, fusobacterium), and candida. *P. aeruginosa*, *S. aureus*, and *P. intermedia* have the ability to produce lipase,[175-177] which can then

cause enzymatic degradation of polyesters, such as polycaprolactone, which is present in Resilon.

AGAR DISC DIFFUSION ASSAY

The agar disc diffusion assay is a relatively new method for analyzing degradation of polycaprolactone and of Resilon. Hiraishi[8] first introduced this method in 2007 when testing for the susceptibility of Resilon to enzymatic degradation. The substance tested is made into an agar emulsion and plated in Petri dishes. Then, the experimental material is applied directly to the agar dish and observed over time for the development of a clear zone depicting diffusion around the test material; in our experiment this would be bacteria.

This method is the opposite of the Kirby-Baur zone of inhibition test developed for testing the effectiveness of various antibiotics against different bacterial species. This antimicrobial susceptibility testing methodology was standardized in 1966.[178] Bacteria are grown on a Petri dish and an antimicrobial agent is applied directly to the agar plate. If substantial antimicrobial activity is present, then a zone of inhibition appears around the test agent, and the size can be related to the level of antimicrobial activity present; the larger the zone, the more potent the agent against that bacterial species.

The diffusion assay applies the same concepts; if the bacteria are able to degrade the Resilon, then a clear zone will be present around the bacteria. The larger the zone, the more efficient and effective the bacteria are at degrading the product. This method is fast, inexpensive, and has the ability to test several bacterial species quickly. However, this method is also dependent on the ability of the agent to diffuse properly though the agar.

MATERIALS AND METHODS

PREPARATION OF RESILON
AND GUTTA-PERCHA EMULSIONS

The Resilon used in this research was manufactured by Sybron Endo. Half a

gram of Resilon was placed into 12.5 ml of chloroform. This mixture was allowed to set

for 24 hours at 37° C until Resilon was completely dissolved. This mixture was

centrifuged with the Beckman centrifuge for six minutes at 3000 rpm at 5°C. The

mixture settled into layers, the heaviest of which was the Resilon settling on the bottom.

The supernatant, residing on the top and consisting of dissolved polycaprolactone, was

decanted. Deionized distilled water containing a phosphoric ester type anionic surfactant

(0.5 g of Plysurf A-210G from Dai-ichi Kogyo Seiyaku Co., LTD; Tokyo, Japan) was

added to the remaining mixture to make a 50-ml emulsion. Zero-point-zero-five (0.05) ml

of 0.1-percent surfactant (Plysurf) was added. This mixture was sonicated for five

minutes with the Branson Sonifier 450. The mixture was left overnight on a stirring plate

with a magnetic stirrer at 40°C under a fume hood, and any remaining chloroform was

evaporated. This remaining emulsion was our stock emulsion of Resilon at a 1.0-percent

concentration. This was calculated as follows: 0.5 g Resilon/50 ml emulsion = 1.0-

percent Resilon. This solution was mixed using an ultrasonic disruptor. Then, 2 g of

BactoTM Tryptic Soy Broth soybean-casein digest medium, 1.5 g Acumedia Agar

Bacteriological, and 90 ml of deionized distilled water were mixed with 10 ml of the 1.0

percent stock emulsion of Resilon to result in 0.1-percent Resilon Agar solution that was

used in the plates. This was calculated as follows: 90 ml water + 10 ml of 1.0-percent

Resilon emulsion = 0.1-percent Resilon concentration. A gutta-percha emulsion was prepared in the exact same manner.

PREPARATION OF RESILON AND
GUTTA-PERCHA AGAR PLATES

The mixture of TSB agar and Resilon emulsion was placed in an autoclave unit (Steris Amsco CenturyTM SV-120 Scientific Prevacuum Sterilizer) at 250 power for 75 min. The resulting mixture was a Resilon emulsion diluted to 0.1-percent. The Resilon agar emulsion was poured into Petri dishes to a depth of approximately 5 mm when 10 ml was used and allowed to solidify. Once solidified, the dishes were stored at room temperature upside down to prevent condensation from settling on the solidified emulsion, so that condensate would form on the lid and could be discarded when opened. The gutta-percha plates were prepared in the exact same manner.

PLATING AND INCUBATING BACTERIAL SPECIES

The microorganisms used were *Prevotella intermedia* (ATCC 25611), *Pseudomonas aeruginosa* PA14 (mutant 3A8 provided by Dr. Gregory Anderson, IUPUI), *Porphyromonas assacharoylitica* (ATCC 25260), *Staphylococcus aureus* (ATCC 6538*), Staphylococcus epidermidis* (ATCC 14990), *Enterococcus faecalis* (ATCC 29212*), Fusobacterium nucleatum* (ATCC 10953), *Staphylococcus mutans* UA159 (ATCC 700610), *Streptococcus sanguis* (ATCC 10556), and *Porphyromonas gingivalis* (ATCC 33277). These were grown in Petri dishes containing Tryptic Soy Agar for 16 h to 18 h in 5.0-percent CO_2 at 37°C in a Model 302 CO_2 incubator. All work with bacteria was performed next to a flame and necessary precautions were taken to prevent cross-contamination between species or contamination from other sources. After

flaming an inoculations loop to sterilize it and cool it back to room temperature, a generous loopful of each of the aforementioned bacterial species were placed onto the solidified Resilon and gutta-percha agar plates. Eight loopfuls of each species were placed onto a single plate spaced evenly apart as to allow room for the clear zone (or halo) measurements if they formed. This was done to determine the variability between samples.

As a positive control, 100 µl of the bacterial enzyme Lipase PS (*Burkbolderia cepacia*) was also placed onto a Resilon agar plate in eight separate locations. It has been shown by Tay[6] that our salivary enzymes are able to degrade Resilon. If clear zones were seen around the lipase, then we would know that the experimental model worked as designed. As a negative control, 100 µl of saline was placed onto the Resilon agar plates in eight separate locations. All plates were then placed in an anaerobic BBL GasPak system incubator container and placed into the Model 302 incubator at 37°C at 5.0-percent CO_2 and allowed to grow for one week. The dishes were checked daily for one week for the presence of a clear halo indicating the degradation of Resilon by the bacterial species.

STATISTICAL ANALYSIS

There was degradation 100 percent of the time with a CI of 95% in select bacteria on the Resilon agar plates. The halos were similar to those seen in the positive lipase control in regards to clarity. No halos were seen at any of the 8 inoculation spots (0%) with a CI of 95% in several of the tested bacterial species on the Resilon agar plates.

RESULTS

After Resilon agar plates with plated bacteria were allowed to incubate for 1 week, the following observations were made. *P. intermedia, P. aeruginosa, P. assacharolytica, S. epidermidis* and *S. aureus* all demonstrated hydrolytic halos, clear zones, at each of the eight inoculation locations (100%, 95%CI 63%-100%) on the Resilon plates. The halos were similar to those seen in the positive lipase control. No halos were seen with *E. faecalis, F. nucleatum, S. mutans, S. sanguis, or P. gingivalis* at any of the eight inoculation spots (0%, 95%CI 0%-37%) on the Resilon plates.

TABLES AND FIGURES

TABLE I

Calculation of Resilon percentage in agar plate*

0.01% Resilon	0.05g Resilon/50ml = .1% Resilon stock solution (RSS)
	10ml RSS+90 ml DW = 0.01% final Resilon concentration
0.05% Resilon	0.25g Resilon/50ml = 0.5% RSS
	10ml RSS+90 ml DW = 0.05% final Resilon concentration
0.1% Resilon	*0.5g Resilon/50ml = 1.0% RSS
	10ml RSS+90 ml DW = 0.1% final Resilon concentration
0.2% Resilon	1.0g Resilon/50ml = 2.0% RSS
	10ml RSS+90 ml DW = 0.2% final Resilon concentration
0.4% Resilon	2.0g Resilon/50ml = 4.0% RSS
	10ml RSS+90 ml DW = 0.4% final Resilon concentration

* Indicates amounts determined to give clearest results.

TABLE II

Results of clear zone test

No clear zone seen	Clear zone seen
F. nucleatum	P. intermedia
E. feacalis	P. assacharolytica
P. gingivalis	S.aureus
S. mutans	S. epidermidis
S. sanguis	P. aeruginosa

Preparation of Resilon and gutta-percha emulsions

1.	Dissolve material in chloroform.
2.	Centrifuge.
3.	Add distilled water and plysurf to supernatant to create an emulsion.
4.	Evaporate chloroform.

Preparation of agar plates

1.	Add TSB, agar, and distilled water to emulsion
2.	 Pour into petri dishes
3.	Plate bacteria and incubate
4.	Observe for clear zone

FIGURE 1. Summary of experimental design.
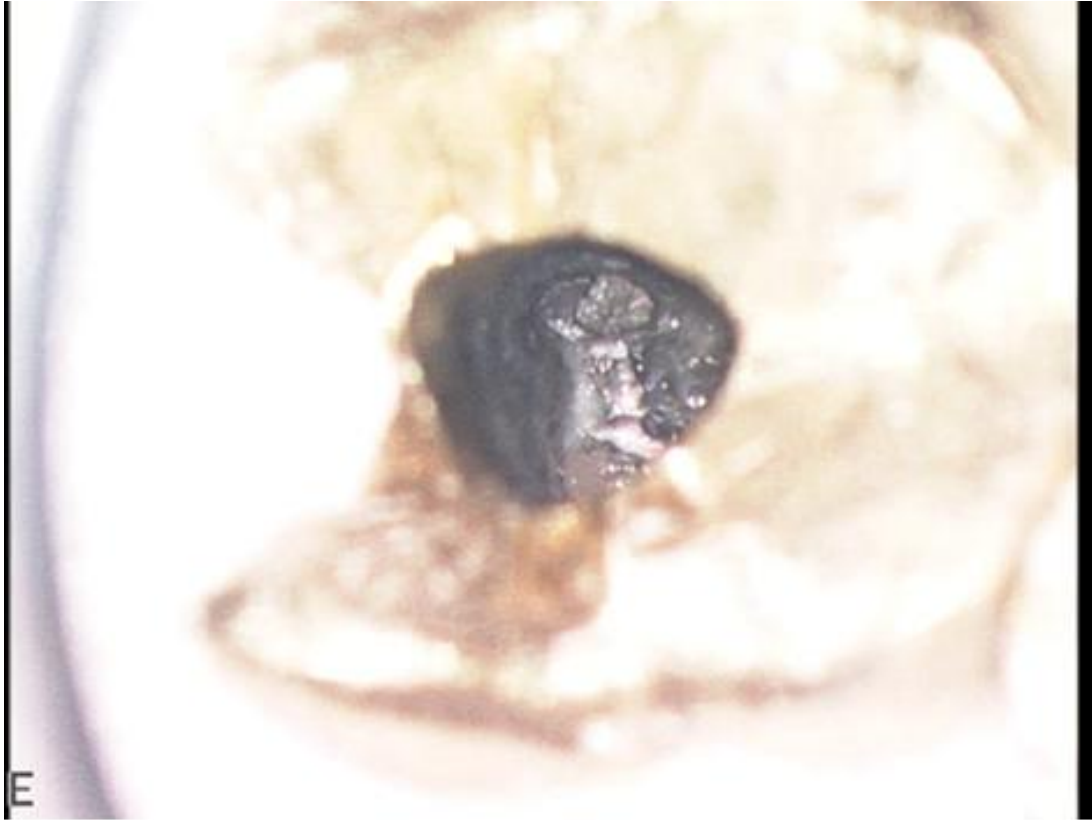
FIGURE 2. Clinical photo of Resilon breakdown within obturated tooth.

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FIGURE 3. Resilon obturation material.
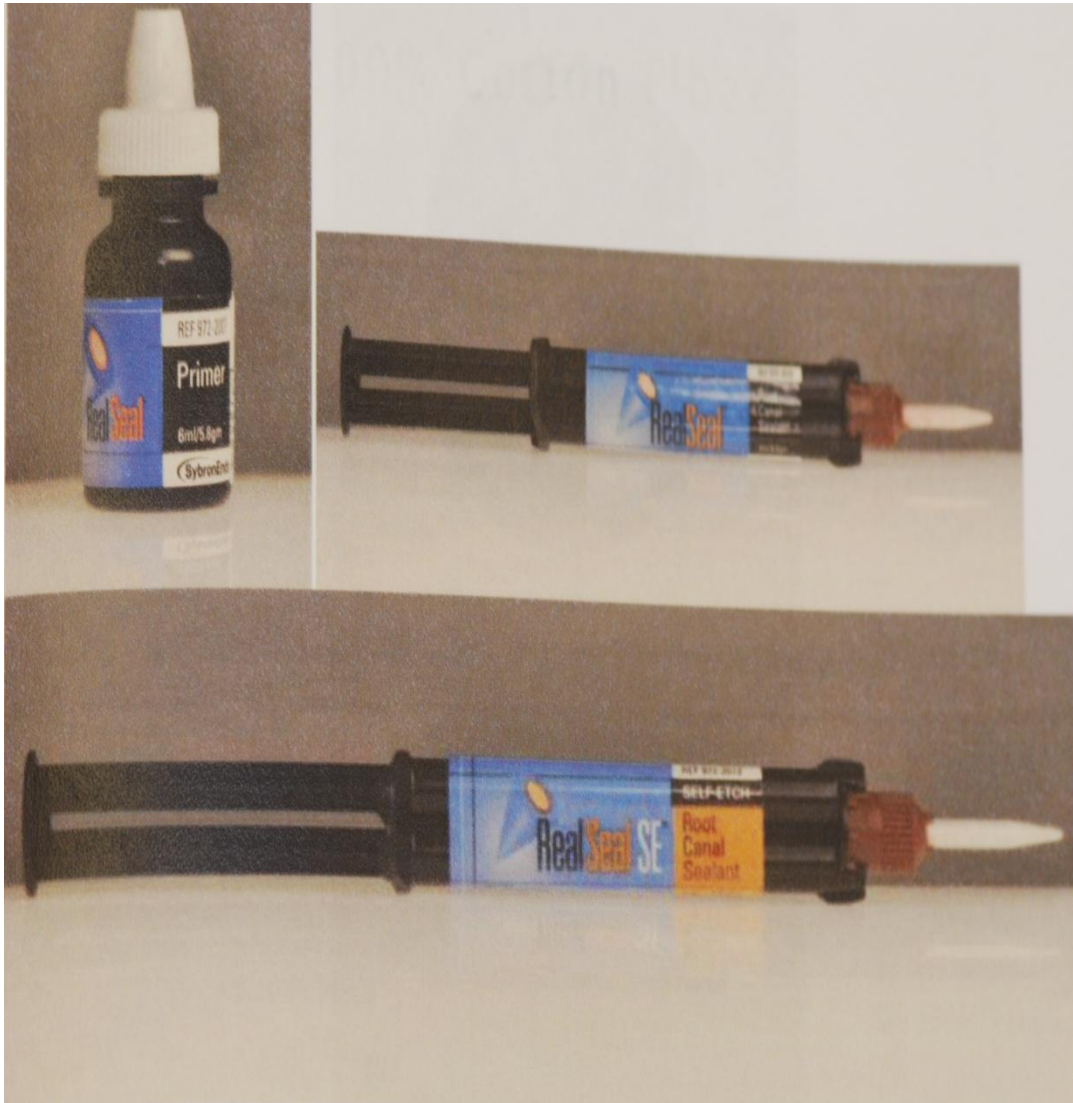
FIGURE 4. RealSeal and RealSeal SE sealers.
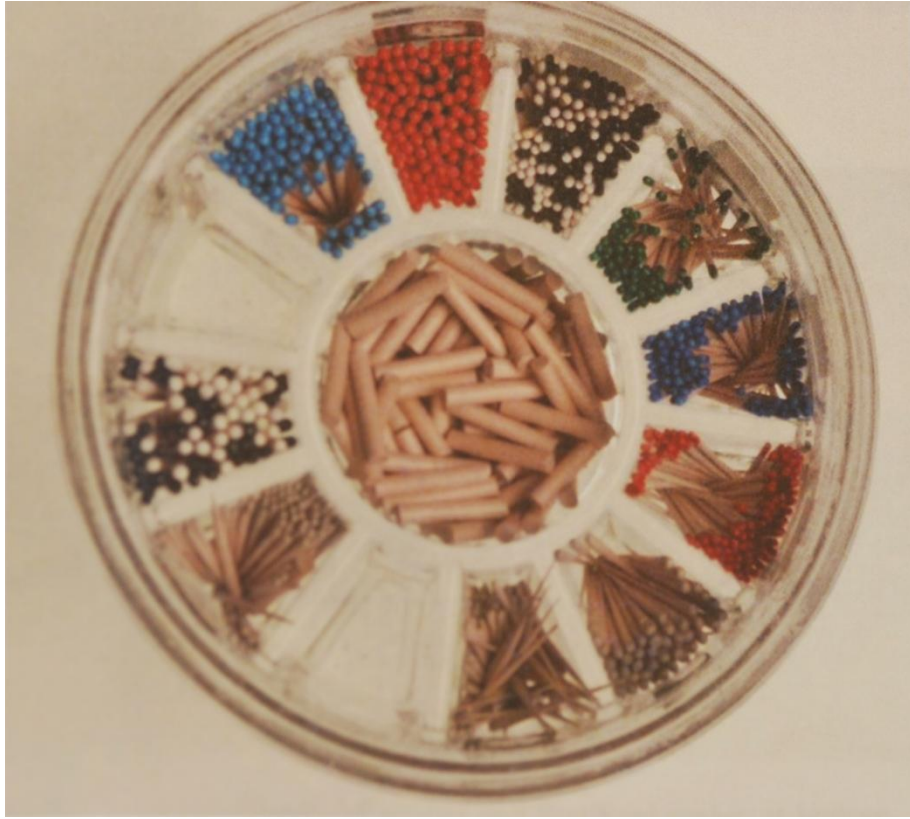
FIGURE 5. Gutta-percha obturation material.

FIGURE 6. Chloroform and Resilon emulsion.
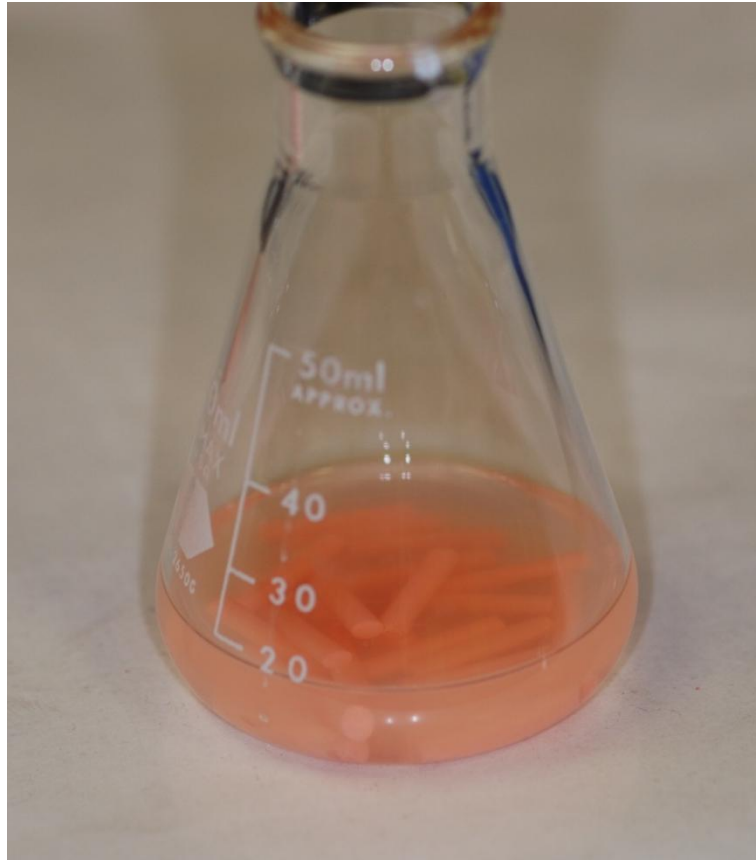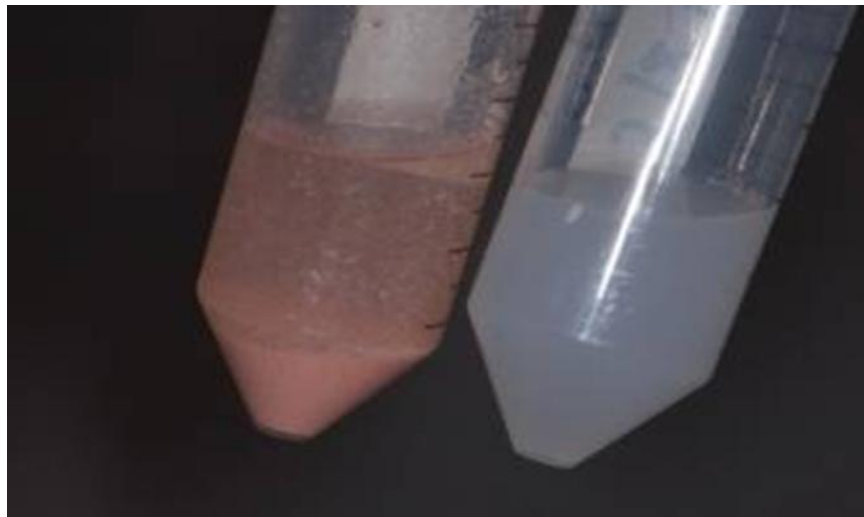
FIGURE 7. Chloroform and gutta-percha emulsion.

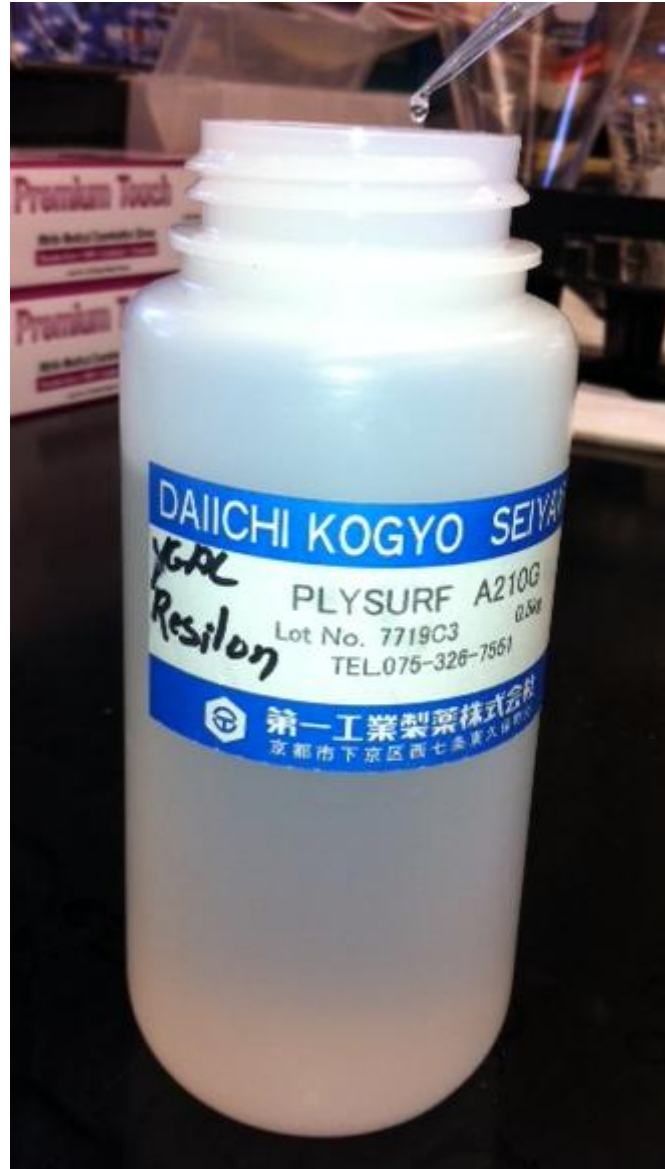FIGURE 8. Centrifuge, separation of supernatant, and prepared emulsion.

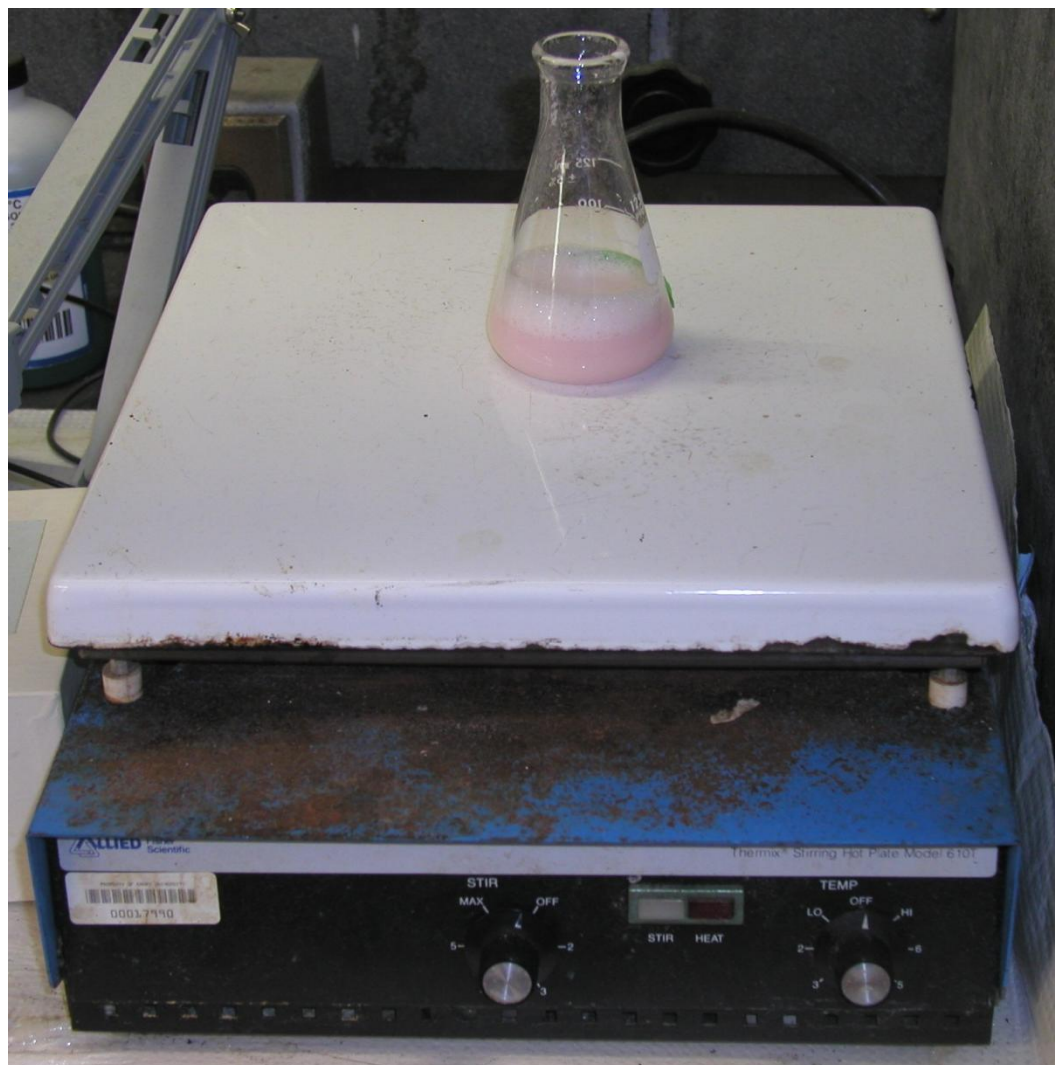FIGURE 9. Plysurf surfactant.

FIGURE 10. Sonicator.

FIGURE 11. Stirring plate with Resilon emulsions to stabilize emulsion
and evaporate chloroform.

FIGURE 12. Tryptic soy broth.

FIGURE 13. Acumedia agar.

FIGURE 14. Autoclave machine.

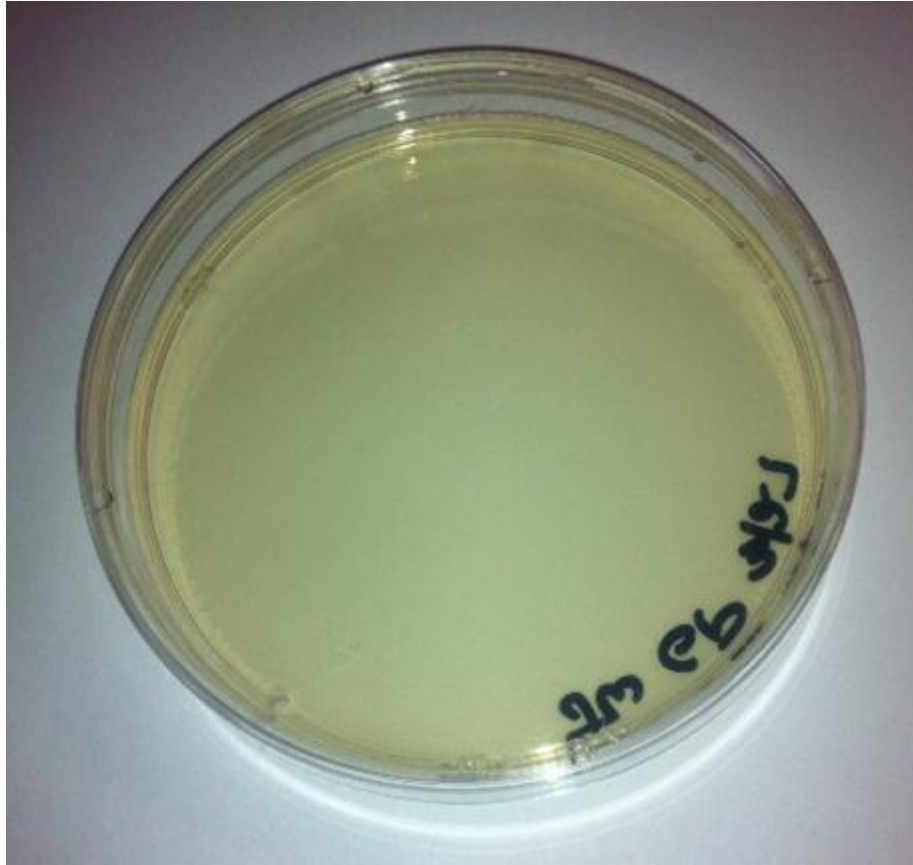FIGURE 15. Petri dish with 0.1-percent Resilon in TSB Agar.

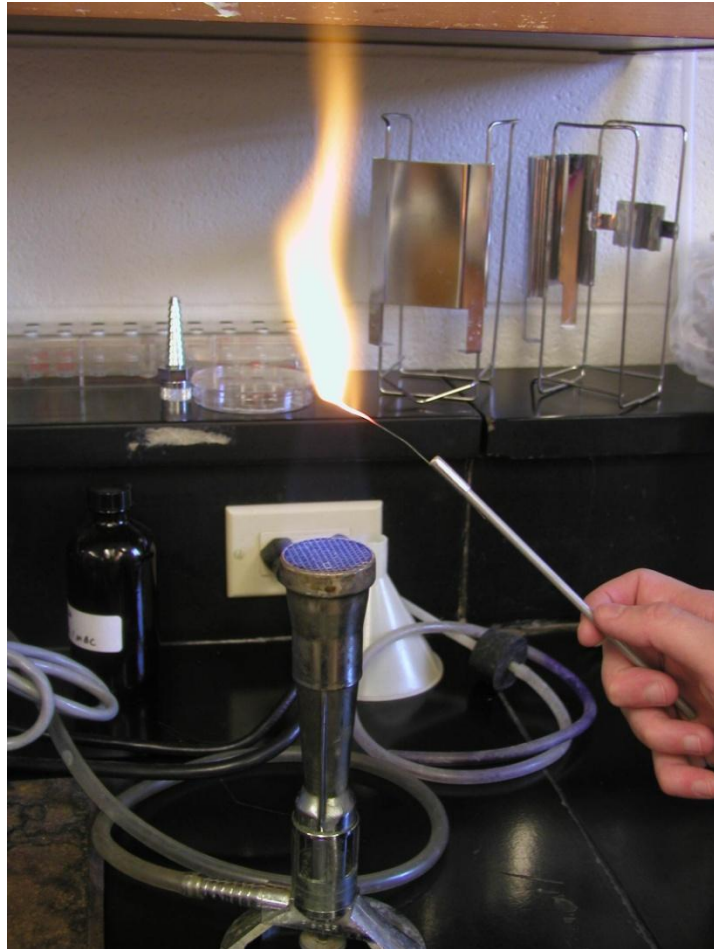FIGURE 16. Petri dish with 0.1-percent gutta-percha in TSB Agar.

FIGURE 17. Flaming the loop.

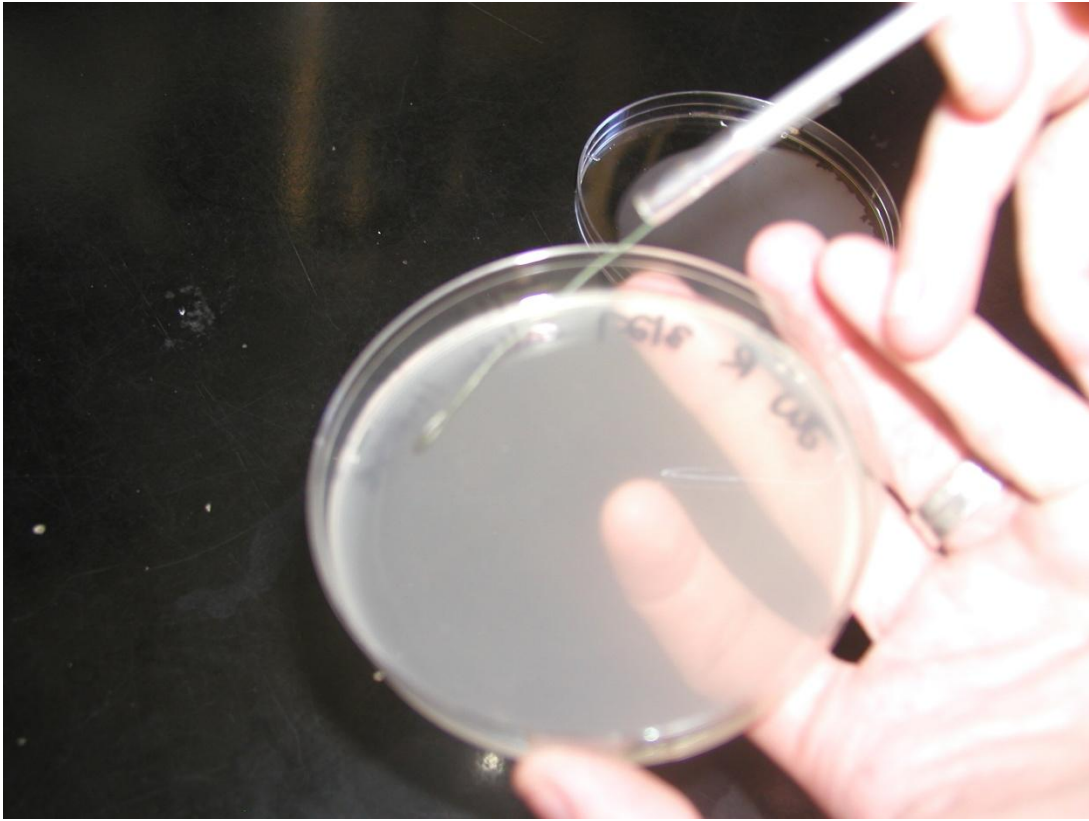FIGURE 18. Gathering loopful of bacteria (*P. aeruginosa*).

FIGURE 19. Plating bacteria onto Resilon agar plate.

FIGURE 20. Anaerobic chamber for bacteria.

FIGURE 21. Incubator.
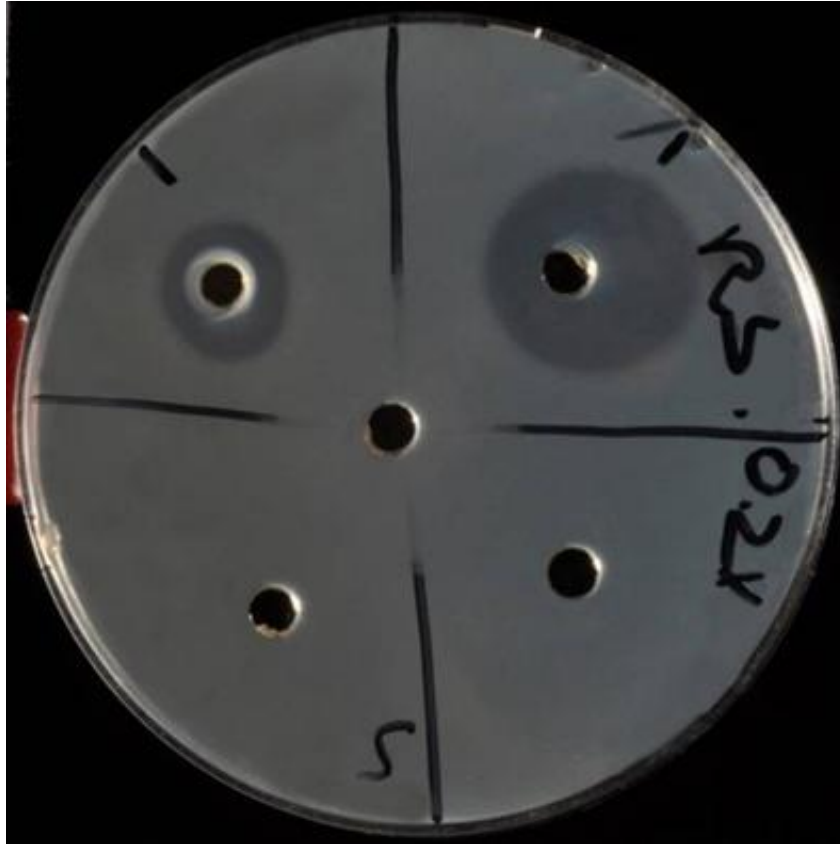
FIGURE 22. Positive lipase control (top row) exhibiting clear zones after 3 days at different amounts. Negative controls, saline, (bottom row) exhibiting no clear zones after 3 days on TSB plate containing 0.2-percent Resilon.
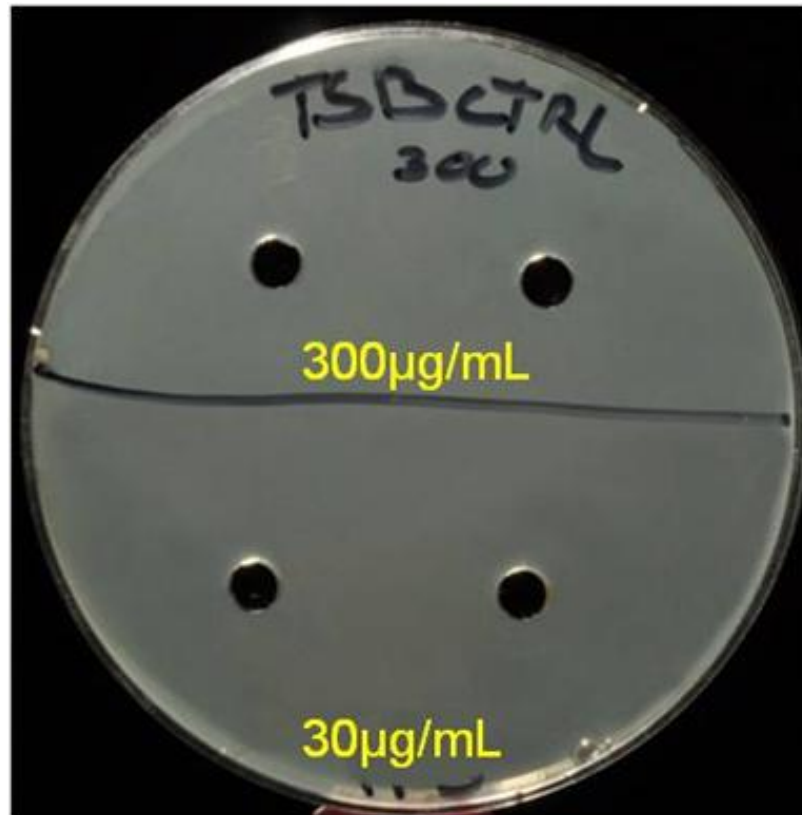
FIGURE 23. Lipase exhibiting no clear zones in TSB plate without Resilon.
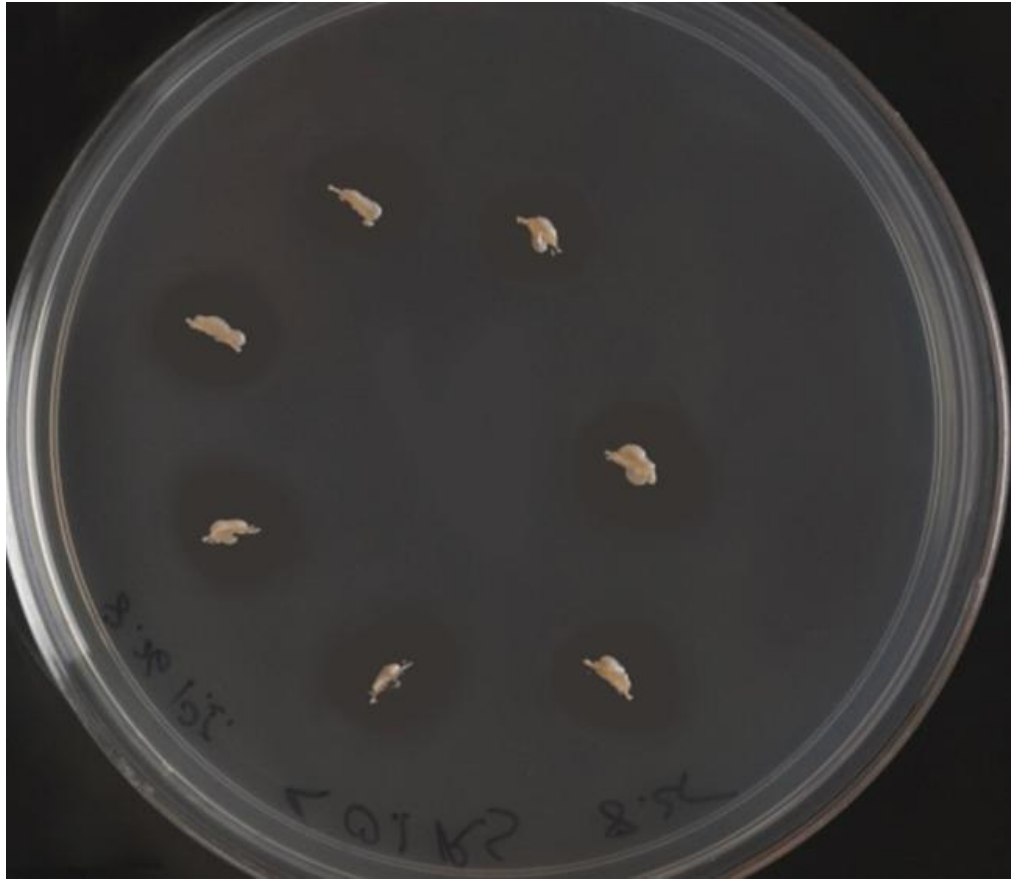
FIGURE 24. *P. intermedia* exhibiting clear zone.

FIGURE 25.   *P. aeruginosa* exhibiting clear zone.

FIGURE 26.   *P. assacharolytica* exhibiting clear zone.
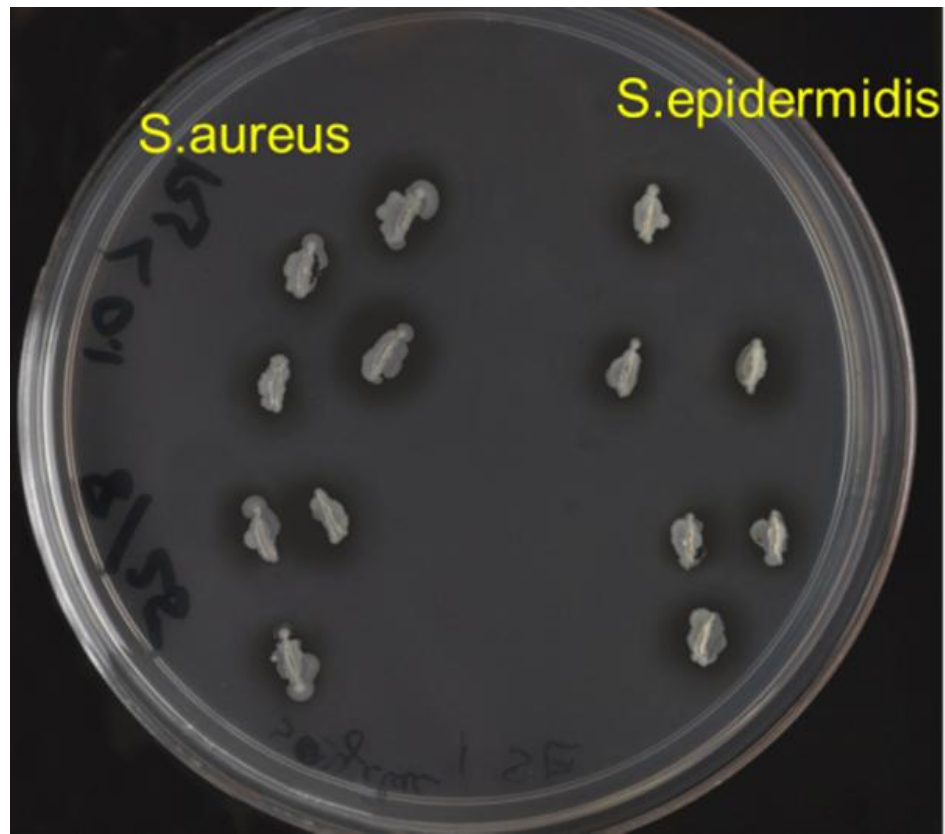
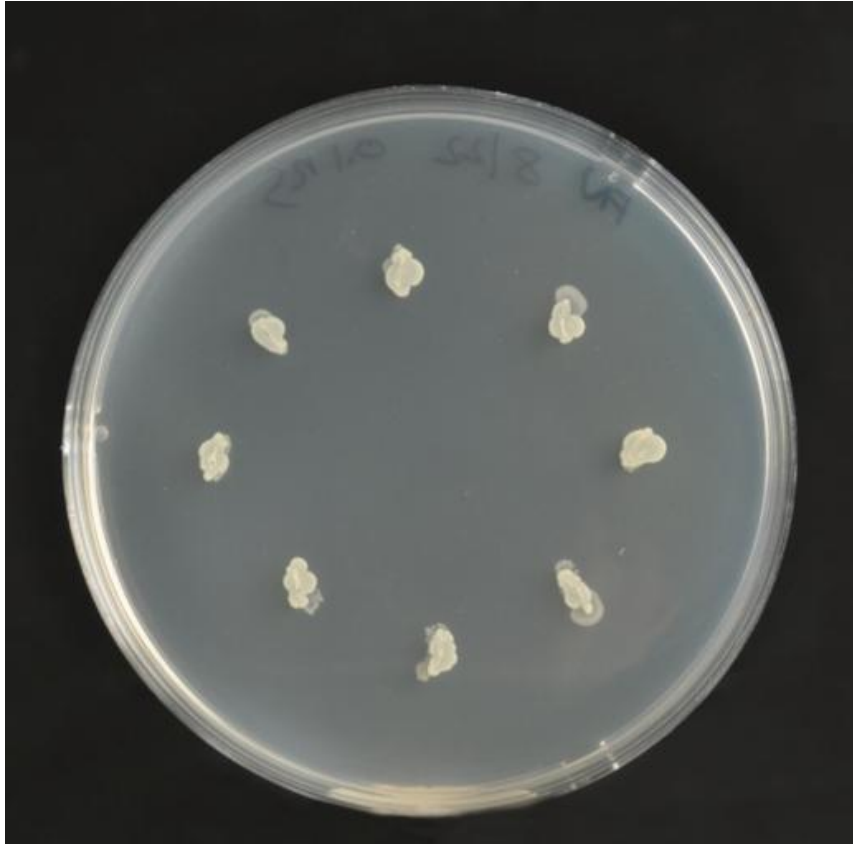FIGURE 27.   *S. aureus* and *S. epidermidis* exhibiting clear zone.

FIGURE 28. *F. nucleatum* exhibiting no clear zone
(similar results seen with *P. gingivalis,*
E. faecalis, S sanguis, and S. mutans).

DISCUSSION

The results of this research prove that Resilon can be degraded by bacteria. Given that the canal cannot be rendered completely aseptic, it is inadvisable to obturate the root canal system with a material that can be degraded by the microorganisms remaining in the canal space. The results of this experiment add to the literature documenting the degradation of Resilon when subjected to dental sludge, bacterial enzymes, alkaline hydrolysis,[6-7, 9] and now, certain bacterial species.

The preparation of the Resilon emulsion had to be altered in many ways from the methods described by Hiraishi for a clear zone to be detected. Determining which concentration of Resilon would give the best results was difficult. Preparations were made in 0.01-percent, 0.05-percent, 0.1-percent, 0.2-percent, and 0.4-percent solutions. In Hiraishi's[8] work a concentration of 0.00032-percent Resilon was used. This was immediately increased to 0.01-percent by using .05 g of Resilon dissolved in chloroform and by following the aforementioned methods, eventually enough distilled water was added to the solution until the mixture was 50 ml total. The addition of TSB, agar, and 90 ml of distilled water to 10 ml of the Resilon stock solution after chloroform was evaporated, and this concentration resulted in plates that were too clear. If a halo were to form, it would not be evident. Therefore, the amount of Resilon was increased 0.25 g. However, it was still too difficult to distinguish if a clear zone was present. The amount of Resilon was increased to 0.5 g, 1.0 g, and 2.0 g, and using either 0.5 g or 1.0 g of Resilon provided the best results. See Table I for calculations of how the desired concentration was calculated.

The next alteration to Hiraishi's method was the deletion of using Whatman's filter paper to remove the inorganic fillers present in Resilon. This did not seem to give a clear solution and resulted in excess material. Decanting the supernatant to remove the inorganic fillers produced a much clearer solution. Also, we did not lose any material employing this method because the layers were separated distinctly after centrifuging.

Even though clear zones were observed using the 0.1-percent and 0.2-percent concentrations of Resilon, the plated emulsion had no color and was translucent in nature. Food coloring was recommended to add to the emulsion prior to pouring it into the Petri dish, so that the results would stand out. However, when food coloring was added, the results were not as distinct. They were blurred by the coloring agent, so this addition was deleted.

Once a method of preparing the Resilon emulsion was established, the next challenge was visualization of optimal clear zones after bacteria were plated. Initially, lapfuls of bacteria would be placed into TSB in sterile test tubes and analyzed for turbidity until the bacterial growth was standardized to an absorbance of 0.5 @600 nm. After this absorbance was reached, 5 μl of each bacterial species were pipetted into wells, which were punched within the Resilon agar plates; however, there were no clear zones observed with this method. Ten ul did not result in the presence of clear zones. Next, the same method for growing bacteria to a standardized absorbance and then placing them directly on the plate without a well was tried, and again no results were seen. This had not captured enough bacteria for degradation to be observed within the agar plate, and active growth was required. Instead of growing the bacteria in TSB, a few colonies of bacterial species were placed directly onto the agar plates, and after one week, there were

slight halos seen around colonies of select species on the Resilon plates. In order to have more distinct zones present, large loopfuls of bacteria were placed directly onto the Resilon plates. Clear zones were distinct after two to three days around several bacterial species indicating a possible dose effect of the amount of bacteria producing more lytic activity.

In addition, even the facultative anaerobes flourished and produced halos in an anaerobic environment. Therefore, in the final protocol, both obligate and facultative anaerobes were placed in anaerobic chambers in the incubator to give the best possible clear zone presence. As with any research, there were many trial runs and alterations to the protocol until a method was developed in which results could be easily and predictably seen. While it was shown that Resilon could be degraded by bacterial species, the large amount of bacteria needed to produce visible results implies that a large amount of bacteria would need to remain within the obturated root canal system, so that the degradation of Resilon could be observed. Also, the observation period could be lengthened to provide sufficient time for the breakdown to be evident as it would be in an infected canal. In the present study, the observation period may have provided larger clear zones in the species in which clear zones were present. However, colonies of each bacterial species able to produce clear zones appeared within the first two days. Those that did not have a clear zone present after one week were considered negative for the ability to form a clear zone. It could be deduced that if clear zones were going to form, they would be evident very soon after plating.

Given that the same bacterial species placed on the Resilon plates were also placed on the gutta-percha plates and that none on the gutta-percha plates produced clear

zones, gutta-percha may not be degradable by the specific microorganisms tested in this study, which are bacteria found within the root canal system.

It is possible that bacterial species remaining within the root canal system may have plenty of nutrients from other sources and will not need to cause biodegradation to Resilon, until the bacteria enter a starvation state after other nutrient sources have been depleted. Growth may have been seen in some of the species, which did not show clear zones if there were no alternate nutrient sources present such as the Tryptic Soy Broth used in the agar plate. This may have forced them to enter a starvation state and search for other potential nutrient sources such as the polycaprolactone present in Resilon. For the purposes of this experiment, select bacteria were shown to degrade Resilon, and future studies may test other bacterial species and biofilm on plates that do not contain TSB.

Often the initial clinical endodontic diagnosis has been pulpitis and subsequently RCT obturated with Resilon has been performed. A few years later, patients are presenting with periapical lesions. Chemical irritants, mechanical irritants, and bacterial byproducts of the inflammatory process can cause pulpitis within the canal system simply by the inflammation present and the bacterial byproducts.[159] How then are teeth without bacteria present initially forming lesions? In previous work done by Tay,[6] Resilon, specifically the polycaprolactone component, can be degraded by enzymes present within saliva. If saliva is introduced at any point during the RCT or during the restorative procedures, or if the apical seal is not ideal, it is possible for Resilon to be broken down over time through exposure to salivary enzymes. It is known that without an adequate seal, bacteria present in saliva have access to the root canal system, and that those

bacterial species or the enzymes they produce have the capability of invading that space and eventually producing a periapical lesion. This would explain why vital cases obturated with Resilon develop lesions at times.

Lastly, it should be noted that three of the five (*P. intermedia, P. aeruginosa,* and *S. aureus*) bacterial species showing the ability to produce a clear zone are also known to produce lipase, which has the ability to degrade Resilon.[175-177] However, *P. assacharoylitica* does not produce lipase and was still producing a halo in the Resilon plate. What gives this bacterial species the ability to degrade Resilon? Are only bacterial species capable of producing lipase or other enzymes in turn capable of degrading Resilon?

Future studies could include Resilon agar plates subjected to additional bacterial species present in primary and secondary infections, as well as biofilm and their ability to degrade Resilon. Experimental groups should include statistically significant amounts of lipase-producing bacteria and possibly other enzymes that may degrade Resilon. It could also be examined whether the lytic activity is secreted from the bacteria or is it only released when the bacteria die?

SUMMARY AND CONCLUSIONS

The purpose of this study was to determine whether select bacteria found within the infected root canal system and in persistent apical periodontitis are capable of degrading Resilon. This was determined using a low concentration of emulsified Resilon and plating it with Tryptic Soy Agar. Once solidified, plates were inoculated with bacterial species and observed for hydrolytic halos or clear zones.

A Resilon emulsion was prepared and dispersed to make 0.1-percent Resilon in 1.5- percent Tryptic Soy Agar plates. Bacterial species were each placed at eight locations on a plate and incubated anaerobically overnight. Gutta-percha was prepared in a similar manner and inoculated with bacteria. Lipase has been shown to degrade Resilon and therefore was used as our positive control.

P. intermedia, P. aeruginosa, P. assacharoylitica, S. epidermidis and *S. aureus* all demonstrated hydrolytic halos at each of the eight inoculation locations (100%, 95%CI 63%-100%) on the Resilon plates, while no halos were observed by these bacteria on the gutta-percha plates. Similar halos were seen in the positive lipase control. No halos were seen with *E. faecalis, F. nucleatum, S. mutans, S. sanguis*, or *P. gingivalis* at any of the eight inoculation spots (0%, 95%CI 0%-37%) on either the Resilon or gutta-percha plates.

The results indicate that select bacteria found in endodontic infections can degrade Resilon but are not able to degrade gutta-percha. This is clinically significant, because bacteria cannot be completely eradicated within the root canal system.

Consequently, obturation materials should not be degradable by the remaining bacteria or by the enzymes they produce.

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ABSTRACT

AN *IN-VITRO* EVALUATION ON THE BIODEGRADABILITY OF RESILON

BY THE MICROBIOTA OF THE INFECTED ROOT CANAL

UTILIZING AN AGAR DISC DIFFUSION ASSAY

by

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Resilon is a resin-based obturation material that claims to create a monoblock through bonding of RealSeal sealer to the dentin walls and to the core material. Resilon is comprised of a biodegradable polymer, polycaprolactone, and inorganic fillers. Resilon has been shown to undergo enzymatic hydrolysis by bacterial enzymes such as lipase. This study aims to demonstrate if bacteria found within the infected root canal system are capable of degrading Resilon utilizing an agar disc hydrolysis method.

A 0.1-percent Resilon emulsion and a gutta-percha emulsion were prepared with Tryptic Soy Agar in plates. Several bacterial species were inoculated in eight spots each on the Resilon and gutta-percha agar plates and the plates were observed for the

formation of hydrolytic halos surrounding bacteria signifying their ability to degrade the material. The bacterial enzyme Lipase PS served as a positive control. *P. intermedia, P. aeruginosa, P. assacharoylitica, S. epidermidis* and *S. aureus* all demonstrated hydrolytic halos, clear zones, at each of the eight inoculation locations (100%, 95%CI 63%-100%) on the Resilon plates. The halos were similar to those seen in the positive lipase control. No halos were seen with *E. faecalis, F. nucleatum, S. mutans, S. sanguis*, or *P. gingivalis* at any of the eight inoculation spots (0%, 95%CI 0%-37%) on the Resilon plates. No hydrolytic halos were seen around any bacterial colonies or the Lipase PS on the gutta-percha plates.

The results of this study indicate that bacteria found in endodontic infections can hydrolize Resilon dispersed into an emulsion. The potential exists for Resilon degradation after its use as an obturation material in infected root canal systems. Given that root canal therapy does not render a canal void of microorganisms, it is prudent to obturate the root canal system with a material that cannot be degraded by bacteria and their enzymes.

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