BIODEGRADABILITY OF RESILON, A RESIN-BASED ROOT CANAL OBTURATING MATERIAL, BY TYPICAL ENDODONTIC PATHOGENS

by

Ashleigh M. Rexford

Submitted to the Graduate Faculty of the School
of Dentistry in partial fulfillment of the requirements for
the degree of Master of Science in Dentistry, Indiana
University School of Dentistry, July 2012.

Thesis accepted by the faculty of the Department of Endodontics, Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

Richard L. Gregory

Anderson Hara

Joseph J. Legan

Susan L. Zunt

Ygal Erhlich

Mychel M. Vail

Kenneth J. Spolnik
Chairman of the Research Committee and Program Director

Date

ACKNOWLEDGMENTS

I feel incredibly blessed to be surrounded by wonderful family, friends, colleagues, and mentors who have helped me through my academic pursuits and throughout my life. I know that I would not be where I am today without them.

I would first like to thank my family for their unconditional love and support. My family has always been there for me to extend a helping hand, offer some words of encouragement, or to tell me to stop being lazy and get done whatever I have needed to accomplish. My parents never settled for less than 100 percent, and I am so grateful. I know that they have made me a better person for it. I feel incredibly blessed to have such an amazing and supportive family.

Dad, thank you so much for always encouraging me and pushing me; you have helped to shape me into the person I am today. Thank you for teaching me that everything you do is a reflection of the person you are. If you halfheartedly do something, from the smallest task to the biggest, you are only letting yourself down. Thank you pushing me to be my best always, regardless of the importance of what I'm doing.

Mom, thank you, for listening to my almost daily phone calls from Boston, for always being there for me, and for your never-ending love and support. You are always there to listen; you have been my rock throughout my years away from home, and I cannot thank you enough. Thank you for being so incredibly supportive and always encouraging me when I felt like there was no way I would ever master a particular thing.

You always have a kind word and when I get frustrated, and you are very good at reminding me not to get so caught up in the details and lose the big picture.

I love you both so much and hope to someday be the kind of parent to my children that you are to me. I will strive throughout my life to continue to live by the values and morals you have taught. Richard and Pam, you have been the best bonus parents anyone could ask for. Thank you for your love and support through this long journey, and for helping to smooth the path for me by being caring and supportive. Brooke, Brittany and Jon, thank you for being wonderful siblings, I know that we have all pushed each other to be our best, and I consider all of you not only family but great friends. I love you guys. There is no way to fully express my gratitude to my family; without all of your love, encouragement and support, I would not be where I am today. I feel truly blessed.

I would like to extend my heartfelt thanks to Dr. Spolnik for accepting me into this program and for giving me this amazing opportunity. Thank you for believing in me when I struggled at the beginning of this program and for always encouraging me over the past two years. You have been a wonderful teacher and mentor, and I am so appreciative of your positive influence in my life. I feel incredibly blessed to be continuing the next phase of my career under your guidance. Dr. Vail, you are an amazing person. I know you are incredibly busy, and yet you have always made time for people, regardless of what might be going on in your life. You seem to do this with ease. I am incredibly appreciative that you are a part of this program, and I look up to you as a mentor and am thankful to have you as a friend. You are a great example of a first-rate clinician and a fantastic person. I am incredibly appreciative of the fact that your door has

always been open to talk about anything; you are a great listener and a wonderful friend. I'm so happy to have had the opportunity to get to know you and look forward to a lifelong friendship.

I am so grateful for the wonderful part-time faculty we have at the school. You make an enormous contribution to our education. Dr. Hine, thank you for always being willing to step in and help, I feel I have learned a lot from you and really appreciate your support and help throughout these two years! I'm convinced there is no canal that you cannot find! Dr. Sahni, your passion for our career is inspiring. Thank you so much for always pushing us to be better, and to strive for perfection; you are a wonderful teacher. Your clinical talent is inspiring, and I will continue to strive to someday have my root canals look as beautiful and consistent as yours. Thank you for never settling for less, for always pushing us to do better. Both of you have had a huge influence on me, and I really cannot thank you enough.

Dr. Adams, thank you for always being positive and encouraging. You are a great example of how important it is to have a positive attitude. Your positivity is infectious; I really have enjoyed learning from you these past two years. Dr. Newton, thank you for your vast knowledge that contributed to my understanding of endodontics, and for your patience and support while teaching in the clinic. Dr. Deardorf, thank you so much for teaching us these past two years. I have learned so much from our case reviews, and I will hear your voice in my head during every consult as you remind me to pick up my probe. You have helped me to find balance by helping me to realize that "the enemy of good is better." Endodontics according to Deardorf has been an invaluable part of my education. Dr. Berman and Dr. Higgins, thank you for your help and dedication in

coming to the school every week; it has been wonderful to have you both teaching. I really appreciate all of your help.

Dr. Erhlich, thank you so much for your tremendous help on my research project. I learned a lot, and you are an invaluable part of the learning process at IUSD. Dr. Gregory – thanks for all your help on my project, and the multiple brainstorming sessions to figure out how to make things work. Dr. Hara, thank you for your help with my project as well. I really enjoyed working with you! Drs. Legan and Zunt, thank you for always being encouraging throughout the past two years. I really appreciate it! Thanks again to all of you for being so incredibly supportive and helpful in this journey; I can't thank all of you enough.

I would also like to extend a special thanks to the staff; your support throughout these two years has been invaluable. I know that I can be very demanding, and I really appreciate all of you putting up with me for the past two years! Elaine, thank you for putting up with my constant changes to the schedule, and for always doing your best to keep me busy. Linda, I have really enjoyed working with you over the past two years and love that you bring in brownies every Friday – thank you! Jenny, thank you for being awesome. I absolutely love working with you! Danyelle, thanks for always being positive; you are a joy to work with, and I know the patients love you! Steve, thanks for always thinking two steps ahead and for always helping me with radiographs. I really appreciate it! Dianne, thank you for always keeping us on top of things and for always being available to help out. Our program is truly blessed to have such a wonderful supportive staff. Your kindness and constant desire to help is greatly appreciated.

And last but not least, I would like to thank my wonderful husband, Alex. I am so grateful that you have been there for me day in and day out, regardless of the circumstances. Thank you for always being supportive, and reminding me to take things one day at a time. You are the love of my life and my best friend. I love you. From the bottom of my heart, thank you.

TABLE OF CONTENTS

Introduction………………………………………………………………………. 1

Review of Literature…………………………………………………………. 7

Methods and Materials………………………………………………………. 42

Results……………………………………………………………………….. 49

Figures and Tables…………………………………………………………. 55

Discussion…………………………………………………………………. 80

Summary and Conclusions………………………………………………… 90

References………………………………………………………………… 93

Abstract…………………………………………………………………… 110

Curriculum Vitae

LIST OF ILLUSTRATIONS

FIGURE 1.	Cutting gutta-percha and Resilon pellets……………………....	56
FIGURE 2.	Samples mounted on ceramic discs with sticky wax………….	57
FIGURE 3.	Finishing of samples………………………………………..	58
FIGURE 4.	Samples measured by profilometry before and after experiment…………………………………………………….	59
FIGURE 5.	Samples sterilized under UV light for 24 hours………………	60
FIGURE 6.	Adding broth to vials………………………………………….	61
FIGURE 7.	Vials with broth……………………………………………….	62
FIGURE 8.	Incubator with samples in incubator………………………….	63
FIGURE 9.	Micropipette used to inoculate samples………………………	64
FIGURE 10.	Broth removed with 10 ml pipette every 3-4 days and fresh broth added to ensure viability of microbial cells…………….	65
FIGURE 11.	Plates of A-H to evaluate uniformity of bacterial species…….	66
FIGURE 12.	Mounted samples…………………………………………...	67
FIGURE 13.	Samples placed in vacuum dessicator for 2 weeks prior to SEM and EDS analysis………………………………………..	68
FIGURE 14.	Comparison of Resilon to gutta-percha following bacterial exposure…………………………………………………….	69
FIGURE 15.	Grading of gutta-percha samples from left to right for gutta-percha………………………………………………………	70
FIGURE 16.	Grading of Resilon samples from left to right………………...	71
TABLE I	Degradation inter-rater agreement……………………………	72
TABLE II	Degradation…………………………………………………	73
TABLE III	Summary of results…………………………………………...	74

INTRODUCTION

The ultimate goal of endodontic treatment is the chemo-mechanical debridement of bacteria from the infected root canal space.[54] In the initial stage, the tooth is opened and the root canal system is accessed. Hand and rotary instruments are used to shape the canal and file the dentin walls. The infected pulp and dentin are removed and then various chemical irrigation solutions are used during the process to dissolve tissue remnants and further disinfect the dentinal walls. In the final stage, the chemo-mechanically cleaned canal space is obturated with a core material coated with a root canal sealer. The access is sealed and the tooth is restored with a permanent restoration.

The objective of obturation is to create a complete seal along the length of the root canal system from the coronal opening to the apical termination. After obturation of the canal, there is evidence that the remaining bacteria and other irritants are entombed by the root canal filling material.[192] Bacteria present within the canal space are the cause of pulpal and periapical infections.[84] Root canal therapy cannot sterilize the canal, and thus the ideal obturating material should possess antimicrobial properties or at least discourage bacterial growth.[192]

Gutta-percha has historically been the gold standard of core obturation materials. Gutta-percha cones have been used successfully with a variety of non-adhesive sealers. Gutta-percha cones consist of: 20-percent gutta-percha (matrix), 66-percent zinc oxide (filler), 11-percent heavy metal sulfates (radiopacifier), and 3-percent waxes or resin (plasticizer).[39] In the past decade, a new core material, Resilon, has been introduced that is challenging gutta-percha. Resilon is a thermoplastic resin composite specifically

designed as a bondable obturating core material. It is used with a dual-cured Bis GMA resin sealer and self-etching primer. This combination was a new approach to sealing the prepared root canal system. Traditional sealers do not bond to the obturating material or to the canal dentin; thus there are gaps that could allow microbial leakage into the canal system. Resilon was developed to attempt the formation of a "monoblock" that would theoretically provide a continuous seal throughout the root canal by chemically bonding the resin sealer to both the obturating material and the root canal dentin.

Resilon is a polymer blend of polycaprolactone, diamethacrylates, mineral fillers and bioactive glass.[95] Resilon is placed after applying a methacrylate-based sealer to a self-etching primer-treated root dentin. This monoblock contains two interfaces. The first layer is between the sealer and primed dentin and the second is between the sealer and Resilon.[183] The bondability of Resilon to resins is attributed to the incorporation of urethane diamethacrylate resin.[82,183] Initial leakage studies of Resilon with Epiphany sealer showed the Resilon seal to be superior to gutta-percha with conventional sealers.[155,187] Further evaluation of Resilon has brought into question the property of Resilon and a methacrylate resin-based sealer's property to create a monoblock due to polymerization shrinkage stresses that cause debonding and gap formation along the periphery of the root canal filling.[183] Additionally, the concentration of the polymeric components, polycaprolactone and urethane dimethacrylates are not optimized for ideal adhesion of Resilon to methacrylate resin-based sealers.[183] In addition to Resilon's lack of creating a complete monoblock, the stability of Resilon as a root canal obturation material has also been questioned.[185]

The thermoplasticity of Resilon is attributed to the incorporation of polycaprolactone.[81] Polycaprolactone is synthetic, biodegradable, semi-crystalline aliphatic polyester that is used in a number of biodegradable and resorbable medical and drug delivery devices.[185] It is susceptible to both alkaline and enzymatic hydrolysis. Previous studies have shown that the polycaprolactone within Resilon is susceptible to alkaline and enzymatic hydrolysis and found that Resilon can be degraded by lipases, microbial hydrolysis and by microorganisms found in dental sludge.[73,184-186] The incorporation of dimethacrylates, mineral and bioactive glass fillers into the Resilon cones did not prevent degradation. It has been suggested that biodegradation of Resilon may occur after endodontic therapy and compromise the success of the endodontically treated tooth.[73,186]

It has been shown that Resilon is not impervious to microbial leakage, especially in regards to the apical seal.[182] Furthermore, residual bacteria thought to be entombed by the obturating material might actually be using Resilon as a biodegradable nutrient source. Tay states that:

> Biodegradability refers to an event which takes place though enzymatic decomposition associated with living organisms. Due to the insolubility of the polymer, constitutional or inducible enzymes are released by microorganisms to depolymerize the biodegradable material before they can utilize the degraded components as carbon sources. It is only during the deprivation of a conventional nutrient source when it is necessary for bacteria to regulate the genes for the transcription of inducible enzymes that are required for the utilization of an alternative carbon source.[184]

The biodegradable material is ultimately converted to water and carbon dioxide under aerobic conditions and/or methane under anaerobic conditions.[184]

Recently, cases of apical periodontitis have been observed in endodontically treated teeth obturated with Resilon. Retreatment of these cases revealed that the Resilon material had actually changed color and had a loss of structural integrity from its initial form.

Apical periodontitis is an inflammatory disease of microbial etiology primarily caused by infection of the root canal system.[192] In recurrent or persistent apical periodontitis, the bacteria and their products either survived the effects of the intracanal disinfection or recontaminated the canal system through coronal leakage.[192]

The microbiota in root canal treated teeth with persistent apical periodontitis is mainly composed of gram-positive facultative anaerobic bacteria in comparison with a primary infection of endodontic origin which is primarily gram negative.[3,37,40,50,64,65,70,106,156,180] This selection process is due to the harsh environmental conditions in the instrumented and medicated canals.[22,65,156,180] Thus a higher occurrence of gram-positive facultative anaerobic bacteria (e.g., streptococci, lactobacilli, *Enterococcus faecalis*, *Olsenella uli*, *Micromonas micros*, *Pseudoramibacter alactolyticus*, and propionibacterium) species are observed.[3,22,50,65,156] In addition, anaerobic bacteria such as *Tannerella forsythia*, *Dialister pneumonsintes*, and *Dialister invisus* were also found in persistent secondary infections.[192]

E. faecalis was chosen for this research project because it is frequently isolated from persistent periapical periodontitis [3,22,33,34,37,50,64,65,70,94,99,106,111,127,136,156,162,175,178,180,208] and has many virulence factors.[33,94,99,121,127,136,175,208] It has been suggested that *E. faecalis* might have an additional virulence factor allowing it to secrete lipase.[33] *Prevotella intermedia* is a black pigmented gram-negative anaerobic rod commonly

found in primary endodontic infections and is also often associated with acute apical abscesses.[156,160] While most microorganisms favoring conditions of a primary endodontic infection are eliminated after chemo-mechanical instrumentation, some anaerobic rods, such as *P. intermedia*, are able to persist.[19,49,141]

Previous studies have shown that the polycaprolactone within Resilon is susceptible to alkaline and enzymatic hydrolysis and can be degraded by microorganisms found in dental sludge.[10,12-14] However, it has not been shown if endodontic bacterial pathogens can degrade Resilon. Furthermore, preliminary experiments have shown that standard Resilon cones exposed to human saliva undergo color change and darken, and this was attributed to the polymicrobial mix found in saliva and not remaining salivary proteins (R.L. Gregory, personal observation). This may suggest that human microbiota affect Resilon and may degrade it. This study aims to examine whether specific bacteria colonizing the infected necrotic root canal can degrade Resilon.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

The toothache has been well documented throughout written history. Recordings of dental disease date back to the 14th century Chinese. They first described the tooth worm theory of dental caries through a character inscription depicting a worm on top of a tooth.[195] Medical writings of the Romans, Greeks, and Chinese from as early as 1500 BC record remedies to treat tooth pain.[77] In 1728 Pierre Fauchard refuted the tooth worm theory and provided accurate descriptions of pulp cavities and canals of various teeth in his book, *The Surgeon Dentist*. Fauchard described treating teeth by opening and draining them for several months followed by placement of lead foil in the chamber.[26]

The first recorded endodontic procedure in this country can be attributed to Robert Woofendale in 1766, who described cauterizing the nerve as well as treating a nerve exposure, in his publication, *Practical Observations on the Human Teeth*. At the end of the 18th century, Fredrick Hirsch was the first to describe diagnosis of a diseased tooth by using a percussion test. In 1809 Edward Hudson is credited as the first person to place root canal fillings into canals in the United States.[26]

Several discoveries led to advances in the field of endodontics. In 1836 pain control was greatly improved by the use of arsenic trioxide to devitalize the pulp prior to removal.[6] In 1884 Carl Koller used cocaine as a topical anesthetic, which lead to even greater improvements in comfort during treatment. The most important discovery in regards to pain control for root canal therapy was in 1905 when Einhorn developed Novocain. Novocain was more effective anesthetic and considerably less toxic than

cocaine. This discovery began an era of painless dentistry, greatly improving the field of endodontics.

In addition to advances in pain control, there were considerable developments in the treatment and obturation of root canals in the 1800s. In 1838 Edwin Maynard is credited with inventing the first broach, as well as several other instruments that aided in the cleaning of canals.[6,119] In 1839 S. P. Hullihen classified causes for toothaches into categories, creating a type of diagnostic system for odontogenic pain.[26] S. C. Barnum invented the rubber dam in 1864, allowing for proper isolation and promoting asepsis.[27] In 1867 G.A. Bowman was the first clinician to use gutta-percha as a sole material for a root canal filling.[6] All of these discoveries helped root canal therapy to gain an increased acceptance in the early 1900s.

One of the most important advances in endodontic technique occurred shortly after Wilhelm Roentgen discovered x-rays in 1895. Edmund Kells quickly saw the advantage of this discovery for the field of endodontics. He used the radiographs to measure a lead wire that he placed in a root canal to determine whether it fit the canal satisfactorily.[55,57,79] In 1913 the first commercially available x-ray machine was introduced, and by 1917 dentists were using radiographs to visualize endodontic procedures and to evaluate success. Many advances in technology have since reduced the exposure times and increased the image quality exponentially.[79]

Endodontic therapy was quickly advancing due to new technologies, materials and techniques. This was leading to increased acceptance for endodontic treatment in the early 20th century. This acceptance however was greatly challenged following a lecture given in 1910 by William Hunter. Hunter condemned dentistry's emphasis on tooth

restoration over extraction, since he believed teeth with caries or infection were "a veritable mausoleum of gold over a mass of sepsis" that he believed led to multiple systemic diseases.[56] Hunter's beliefs lent further support for the focal infection theory. This theory resulted in the needless extraction of millions of pulpless and healthy teeth to prevent focal infection, as well as the discontinued teaching of endodontics in some schools.[57]

In the late 1930s dentists such as Edward C. Kirk and C.N. Johnson began to speak out against the wholesale extraction of all pulpless teeth.[55,56] In 1937 Logan demonstrated that the presence of microorganisms did not necessarily imply the presence of infection, and that some bacterial presence is normal.[12] This was one of the major contributions in highlighting the lack of clinical evidence to support the focal infection theory. In the late 1940s laboratory research and clinical evidence was sufficient to confirm that devitalized teeth are not a causative factor of systemic disease.[58] As the focal infection theory fell out of favor, endodontic treatment began to steadily gain acceptance in the dental and medical communities.

Growing acceptance of root canal treatment led to the development of a specialty within dentistry limited to root canal treatment. The term endodontics was coined by Harry B. Johnson who created the first practice "limited to endodontics." In 1943 a group of 20 dentists met to create an organization of endodontists. This is now known as the American Association of Endodontists. In 1946 the first *Journal of Endodontics* was published and in 1956 the American Board of Endodontics was established. Due to the growth and development of the field, in 1963 the ADA officially recognized endodontics as a specialty area of dentistry.[12]

THEORY OF ENDODONTICS

Arguably the most important study to the field of endodontics was performed by Kakehashi et al[84] in 1965. The results of this study showed that the development of apical periodontitis cannot occur without the presence of bacteria.[84] Other studies support this finding and provide strong evidence that indicates microbial infection is essential for the development and progression of apical periodontitis.[13,107,179] More recent research has found that in addition to bacteria; fungi, archaea and viruses have also been found in association with endodontic infections; however it is unknown how these microorganisms may contribute to apical periodontitis.[140,163,166,197] In 1967 Schilder[149] stated that the ultimate goal of endodontic therapy is to eliminate the root canal system as a source of infection and inflammation to the periapical tissues. This is achieved by cleaning and shaping and finally closing the root canal system by way of a compact three dimensional hermetic obturation that acts as a barrier between the root canal system and the tissues surrounding the tooth.[149] Thus, the ultimate goal of endodontic treatment is either to prevent the development of apical periodontitis or to create adequate conditions for periradicular tissue healing.[156]

In 1955 Stewart[174] described three specific phases required for successful endodontic therapy: chemo-mechanical preparation, microbial control, and obturation of the root canal. He emphasized the importance of each step, but considered that chemo-mechanical preparation was most influential for success. He showed that as the root canal system is enlarged, the amount of viable microorganisms decreases. Larger size and increased taper also allowed for increased volume of irrigation solutions.[174] While elimination of the bacteria through chemo-mechanical preparation is a huge factor in the

outcome of endodontic therapy, Kuttler[86] argues that proper obturation is at least as important as the chemo-mechanical step, if not more. He believed that a hermetic seal can create an environment leading to the formation of healthy periodontium, normal osseous structures, and intact lamina dura.[86]

Grossman[59] stated 13 principles to be followed in every root canal procedure to ensure the highest rate of success. These are the following:

1) Aseptic technique should be followed.
2) Instrumentation should be confined within the root canal.
3) The root canal should be entered by a fine, smooth canal instrument, and never forced apically.
4) Biomechanical instrumentation is required to enlarge the canal space from its original size.
5) Irrigation of the canal with antiseptic solution during instrumentation is required in order to serve as a lubricant, diminish the amount of microorganisms, and to facilitate the removal of dentinal shavings.
6) The antiseptic solution should remain within the canal space and be non-irritating to periapical tissue.
7) Fistulas will heal without special treatment.
8) Negative culture should be obtained prior to obturation.
9) Canal must be hermetically sealed during obturation.
10) Obturation material should be non-irritating to periapical tissues.
11) For an acute alveolar abscess, drainage must be obtained, whether through the tooth or incision.

12) Avoid injecting into the area of infection to prevent the spread of microorganisms into deeper tissue.

13) Root surgery may be needed in the treatment of some pulpless teeth.

The principles came to be known as Grossman's tenets and are still advocated today to evaluate new technologies.

In 1995 Ray and Trope[133] examined 1010 endodontically treated teeth restored with permanent restorations. They concluded that the quality of the coronal restoration was significantly more important than the quality of the endodontic treatment in the presence of apical periodontitis. An epidemiological study that correlates the importance of the coronal seal with retention of teeth is the Salehrabi and Rotstein[143] study that followed 1,126,288 patients over a period of 8 years. In this study, 85 percent of failures lacked a coronal restoration. A systematic review was done by Gillen et al.[44] that found the odds of healing of apical periodontitis increase significantly if the tooth has both adequate root canal therapy and restorative treatment as opposed to inadequate restorative treatment. Although many investigators argue on which step is most influential in the success of endodontic therapy (chemo-mechanical debridement or the creation of a hermetic seal) they agree on the necessity for a good coronal seal.

SUCCESS OF ENDODONTIC THERAPY

It is important for endodontists to be well-versed in the success rates of nonsurgical and surgical treatment to make evidence-based decisions while suggesting endodontic treatment options. Success rates of endodontic treatments have been well documented, and the major indicator of successful therapy is the absence of clinical symptoms and periapical lesions. Success has classically been evaluated by using clinical

signs and symptoms, radiographic interpretation, and histopathologic evaluation of excised tissue.[87]

Historically and currently, a question exists about whether treatment success rates for teeth with periapical periodontitis are affected by the number of visits. In 2005 Sathorn et al.[146] made a systematic review and meta-analysis of single- versus multiple-visit endodontic treatment and found that single-visit endodontic therapy was slightly more effective than multiple-visit. Another notable finding of this study showed that differences in healing rates between these two regimens were not statistically significant. Conversely, Figini et al.[38] in their Cochrane systematic review found no detectable difference in the effectiveness of root canal treatment between single and multiple visits. However, they also found that patients undergoing a single visit might experience a slightly higher frequency of swelling and require more analgesic use. A systematic review done by Su et al.[177] in 2011 discussed differences in healing rate and post-obturation pain of single- versus multiple-visit endodontics and found no significant difference in the healing rate; but, they did find that patients experience less post-obturation pain after single-visit endodontics. In conclusion, while there are slight differences reported in discomfort between the two regimens, overall the literature supports that no significant difference overall is found in the healing outcomes of single- versus multiple-visit endodontics.[38,177]

In 1990 Sjogren et al.[164] evaluated success rates of primary endodontic treatment. These researchers evaluated long-term factors affecting the results of treatment that were directly dependent on the preoperative status of the pulp and periapical tissue. Success rates regardless of vitality were 96 percent if there were no preoperative periapical

lesions. If a lesion was present, success rates dropped to 86 percent. Another factor affecting success rate was the length of obturation. If the obturating material was 0 mm to 2 mm from the apex, then the success rate was 94 percent; if the material was past the apex, the success dropped to 76 percent, and if the material was greater than 2 mm short of the apex in a necrotic tooth with a lesion, the success rate dropped to 68 percent. In a systematic review, Shafer et al.[147] reiterated that obturating 0 mm to 2 mm short of the apex as opposed to past the apex resulted in a 28.8-percent improvement in success rate, which was found to be significant.

Considering epidemiological studies, Lazarski et al.[87] showed that 94.44 percent of non-surgically treated teeth remained functional over an average follow-up time of 3.5 years. The data was composed of 110,766 nonsurgical root canal procedures completed by endodontists and general practitioners. Another finding of this study was that teeth that were not restored after root canal treatment were significantly more likely to be extracted. Salehrabi and Rotstein[143] conducted one of the largest epidemiological studies concerning survival of endodontically treated teeth. They reviewed 1,126,288 patients and 1,462,936 teeth from all 50 states over a period of eight years. Ninety-seven percent of all teeth were retained for eight years. The combined incidence of teeth needing retreatments, apical surgery, or extraction was only 3 percent. Another notable finding of this study was that 85 percent of teeth requiring extraction lacked a coronal restoration. Another noteworthy observation from a systematic review by Stavropoulou[173] with regard to the survival of root canal treated teeth found that survival rate is significantly higher if the tooth has full coronal coverage as opposed to a direct restoration (resin

composites, amalgam, cements). These findings further support the conclusions of Ray and Trope[133] and Gillen et al.[44]

In 2011 Ng et al.[112] found that 4 preoperative factors, 6 intra-operative factors and 1 post-operative factor were found to be significant indicators for success. Pre-operative factors of significance were: periapical lesion, size of lesion, presence of a sinus tract, and root perforation, which if present, all lowered odds of success. Intra-operative factors of significance to increase the odds were achieving patency; while an uninstrumented canal (short of terminus), long filling, use of 0.2-percent chlorhexidine, and a flare-up reduced odds of success. For retreatments 17-percent EDTA was shown to increase success two-fold. A sound coronal restoration increased the odds of success eleven-fold. An additional factor to be considered when restoring an endodontically treated tooth that requires a post is the type of post utilized. A literature review by Cagidiaco et al.[21] of two randomized controlled trials indicated that fiber-reinforced composite posts outperform metal posts in the restoration of endodontically treated teeth.

INSTRUMENTATION

The goal of endodontic treatment is to promote healing of periapical pathology as well as preventing periapical pathology to develop. One of the most important phases of endodontic therapy is maintaining the original anatomy of the canal during root canal preparation.[148,174] This step involves proper removal of vital and necrotic tissues from the root canal system as well as systematic enlargement of individual canals for removal of infected dentin. Proper cleaning and shaping of canals allows access for irrigation solutions and medicaments to remove debris and disinfect the root canal system, therefore eliminating infection.[75]

Currently, a multitude of instruments are available that aid in achieving the mechanical goals of endodontic therapy. They can vary in length, taper, tip design, and type of metal they are composed of. Hand instruments include files, broaches, reamers, and Hedstroms. They have been used for decades and are still an invaluable part of the armamentarium needed for proper instrumentation. Hand instruments typically have 16 mm of cutting surface and are standardized in size. Previously, carbon steel was the material of choice for hand instruments, but currently stainless steel is used because it does not corrode, thus greatly improving the quality of these instruments.[63]

The K-type file is the oldest useful instrument for cutting dentin. Fabrication of a K-file is done from a wire that has been ground into the shape of either a square or triangle and then twisted to create a file or a reamer. A file has more flutes per length unit than a reamer. These files are used for penetration and enlargement of canals. It is advantageous to use this file in a reaming motion due to the fact that this motion causes the instrument to self-center in the canal. In contrast, when used in a filing motion, K-files readily cause transportation of the canal.[103]

H-type instruments are ground from a tapered blank and have spiral edges arranged to only allow cutting on a pulling stroke. This type of instrument is more efficient at cutting due to a positive rake angle. The Hedstrom file is the most popular H-type instrument, and is excellent for removing bulk dentin when used in a filing motion. While Hedstroms are very efficient for removal of dentin, their aggressive nature also leads to a higher frequency of procedural accidents.[103]

A major limitation of stainless steel hand instruments is in preparation of curved canals. As a stainless steel files increase in diameter, it becomes less flexible and more

likely to transport the canal system. Roane[135] stated that the goals of endodontic treatment are to "remove the canal's soft tissue contents as completely as possible, eliminate as completely as physically possible any microbial elements, and create a situation within the canal system to the apical supportive structures." Roane explained that small-preparation diameters are not ideal, due to reduced cleansing of the canal space. Roane created a balanced force technique to allow for proper instrumentation of curved canals with stainless steel files. His technique involved winding a K-file to place with a light clockwise rotation, then turning the file counterclockwise while exerting apical pressure on the file. The balanced force technique allowed negotiation of curved canals without transportation or ledge formation.

In 1988 Walia et al.[201] published a study that provided a major breakthrough in endodontic instrumentation. It was observed that nickel-titanium files had two to three times the elastic flexibility of stainless steel files as well as a superior resistance to fracture. These properties allow for more precise instrumentation and preparation of the apical region of the canal, especially in curved canals.

Nickel-titanium files were later modified to be used as rotary instruments. In 1995 Esposito and Cunningham[35] demonstrated that nickel titanium files were significantly more effective in maintaining the original canal path when compared with stainless steel rotary instruments. Currently, several nickel-titanium rotary instruments are available in differing shape, cross-section, and tip design.

Mechanical preparation of the root canal is an invaluable step in endodontic therapy, but it is important to remember it is only one step of several in obtaining a properly cleaned, shaped, and obturated root canal system. In 2001 Spangberg[170]

concluded that without a superior knowledge of root canal anatomy and pathology, mechanical instrumentation will not enhance endodontic outcome. Mechanical instrumentation alone does reduce the number of bacteria; however, it still leaves a significant amount of dentin surface area untouched.[126] Thus, mechanical instrumentation alone is not sufficient to deem a canal disinfected.[159]

IRRIGATION SOLUTIONS

Mechanical instrumentation alone cannot achieve an adequately clean root canal. Siqueria et al.[159] demonstrated that although nickel titanium instrumentation significantly reduced the bacterial load within the root canal system, regardless of instrumentation technique and file sizes used, bacteria were never eliminated from the root canals. A chemo-mechanical preparation of the root canal space is needed to eliminate bacteria located inside dentin tubules, fins, and other ramifications commonly unaffected by instrumentation alone.[103] In addition to cleaning the canal of debris, irrigation solutions increase efficiency of instruments by acting as a lubricant.[1]

In the 1940s water was a common irrigation solution due to cost effectiveness and availability. In 1981 Bystrom and Sundqvist[20] demonstrated that when saline was used as an irrigation solution with mechanical instrumentation, a significant amount of bacteria persisted within the root canal system. These results led to the utilization of more effective irrigation solutions. In 1984 Harrison[72] described key properties of an irrigation solution: antimicrobial activity, dissolution of necrotic tissue, prevention of smear layer formation or dissolution of smear layer, inactivation of endotoxin, and biocompatibility. Currently, no irrigation solution meets all of these requirements.

The gold standard and the most common irrigation solution used today is sodium hypochlorite (NaOCl).[71,105,193] It is available in a variety of concentrations and is a potent antimicrobial agent, and at increasing concentrations effectively dissolves pulpal remnants and organic portions of dentin.[71] NaOCl has a long shelf life, is inexpensive, has a lubricating and bleaching action, and is available in commercial 5.25-percent solutions. Naenni et al.[110] evaluated the soft tissue dissolution of several irrigation solutions and concluded that NaOCl was the only irrigation solution with any significant capacity to dissolve tissue. Harrison[72] concluded in his study that 5.25-percent NaOCl concentration is the irrigation solution of choice and any dilution from that decreases its effectiveness.

Although NaOCl is a potent antimicrobial agent and effective tissue dissolvent, it also has undesirable effects. NaOCl causes cytotoxicity and caustic effects on healthy tissues even at low concentrations. Pashley et al.[122] concluded that all concentrations of NaOCl are cytotoxic, causing complete hemolysis of red blood cells. Several studies have been published which document the morbidity associated with NaOCl accidents, which presents as an immediate tissue response causing severe pain and inflammation.[11,32,42]

In addition to NaOCl's cytotoxicity, other weaknesses of this irrigation solution include resistance of certain microorganisms to its antimicrobial effects as well as its lack of ability to completely remove the smear layer. Gomes et al.[48] observed that it took a 5.25-percent concentration over a time period of at least 30 seconds to completely eliminate *E. faecalis*, and as the percentage of NaOCl decreased, the contact time required significantly increased, with 0.5 percent requiring 30 minutes. Radcliffe[130] confirmed the higher resistance of *E. faecalis* to NaOCl with his research findings. These

disadvantages have led to investigations of alternative irrigation solutions, to enhance both the cleaning and antimicrobial properties for increased endodontic success.

The use of chlorhexidine (CHX) in dentistry has been advocated because it is antimicrobial, has substantivity, and is well tolerated by tissues.[103] CHX bas a broad antimicrobial spectrum and is active against gram-positive and gram-negative bacteria, facultative anaerobic and aerobic bacteria, spores, viruses, and yeast.[29] CHX is limited to two concentrations, 0.12 percent and 2 percent, both of which have an extremely low level of tissue toxicity locally and systemically.[92] Jeansonne and White[80] evaluated the antimicrobial properties of 2.0-percent CHX with 5.25-percent NaOCl and found no significant difference in regards to antimicrobial effect. Conversely, Oncag et al.[116] and Vianna et al.[198] showed *in-vitro* CHX to be superior to NaOCl in killing *E. faecalis* and *Staphylococcus aureus*. Additionally, Ohara et al.[115] investigated the antibacterial effects of six irrigation solutions against anaerobic bacteria and reported that CHX was most effective.

CHX has been shown to exhibit substantivity in root dentin. Leonardo et al.[90] investigated the antimicrobial activity along with the substantivity of 2.0-percent CHX in an *in-vivo* experiment. The authors concluded that CHX prevents antimicrobial activity *in vivo* for up to 48 hours. An *in-vitro* study conducted by White et al[204] demonstrated antimicrobial effects of 2.0-percent-CHX-treated teeth lasting the entire 72-hour testing period. Rosenthal et al.[138] continued research on the substantivity of CHX and found that CHX is retained in root canal dentin in antimicrobially effective amounts for up to 12 weeks.

Like sodium hypochlorite, chlorhexidine also had its disadvantages. CHX is dependent on the pH, which is greatly reduced in the presence of organic matter and lacks tissue solvency.[139,206] In 2006 Clegg[24] demonstrated that CHX was unable to affect biofilm structure, while NaOCl was shown to completely remove biofilm. However, when CHX is coupled with the use of a surfactant (cetrimide) it was found to be efficacious against biofilms.[7] In 2008 Estrela et al.[36] did a systematic review on the efficacy of NaOCl and CHX against *E. faecalis* and found that both showed low ability to eliminate *E. faecalis* when evaluated by either PCR or culture techniques. Despite these limitations, significant evidence supports use of CHX as an adjunct to NaOCl rather than as a single irrigating solution.

Another limitation of NaOCl is that it only dissolves organic matter. The smear layer in the root canal is composed of both organic and inorganic debris. Research has shown the smear layer to harbor bacteria and yeasts in infected canals, and its removal could increase the success of endodontic therapy.[60] Completely removing the smear layer facilitates the cleaning and elimination of infected tissue and thus contributing to the removal of bacteria in the root canal system. In 2007 Shahravan et al.[153] concluded that smear-layer removal improves the fluid-tight seal of the root canal system, whereas other factors such as the obturation technique or the sealer itself did not produce significant effects. The results of this systematic review emphasize the importance of complete smear layer removal.

Ethylenediamine tetra-acetic acid (EDTA) is an irrigation solution that was first used in endodontics in 1957.[103] EDTA is a disodium salt that chelates calcium ions leading to the decalcification of the smear layer along the canal walls and increases the

diameter of the dentinal tubules.[10] It has been shown that EDTA-facilitated smear layer removal improves the antimicrobial effect of various other irrigation solutions in deeper layers of dentin.[118] Niu et al.[114] investigated effects of EDTA alone and EDTA in conjunction with NaOCl and found that more debris was removed by irrigation with EDTA followed by NaOCl than with EDTA alone.

In conclusion, the chemical component of root canal debridement is an invaluable step towards successful endodontic therapy. NaOCl, CHX and EDTA have all been shown to be advantageous to utilize during debridement of the canal, and that a combination of solutions rather than one single irrigation solution is most efficacious in the chemo-mechanical debridement of the root canal space.[134,207]

ENDODONTIC MICROORGANISMS AND INFECTION

A landmark study in the field of endodontics was done in 1965 by Kakehashi et al.[84] that conclusively established the critical relation between bacteria and pulpal infection. This study showed that periapical infection did not occur without the presence of bacteria, despite pulpal exposure. In 1976 Sundquist[179] reinforced the concept that pulpal pathosis 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, where as periapical pathosis was not present in any necrotic tooth with intact crowns in a sterile environment. In 1981 Moller[107] demonstrated clinically and radiographically that infected pulp tissue in primates displayed inflammatory reactions. Thus the key determinant in endodontic success and healing is the elimination of bacteria.

The inflammatory response in the pulpal tissue is activated on a local level by bacterial antigens before microorganisms even reach the pulpal tissue.[14] Before bacteria

are able to reach the pulp, the inflammation might be reversible. However, once a portal of entry is available into the pulp cavity, bacterial infiltration through the dentinal tubules will result in pulpal necrosis. Microorganisms are able to invade into and persist in the inner third of the dentinal tubules.[91] Bacterial species vary in numbers and types throughout an infected root canal, and bacterial populations within root canals vary depending on the type of infection, primary or persistent.[156]

Bacterial species often seen in primary infections belong to a diverse genera of gram-negative (fusobacterium, dialister, porphyromonas, prevotella, tannerella, treponema, campylobacter, and veillonella) and gram-positive (parvimonas, filifactor, pseudoramibacter, olsenella, actinomyces, peptostreptococcus, streptococcus, propionibacterium, and eubacterium) bacteria.[160] Conversely, the microbial flora of secondary or persistent periapical periodontitis is mainly composed of gram positive facultative anaerobic bacteria.[3,37 40,50,64,65,70,106,156,180] This selection process is due to the harsh environmental conditions in the instrumented and medicated canals.[22,65,156,180] Due to this selection process, an increase of gram-positive facultative anaerobic bacteria species (e.g. streptococci, lactobacilli, *E. faecalis*, *Olsenella uli*, *Micromonas micros*, *Pseudoramibacter alactolyticus*, and propionibacterium) is observed.[3,22,50,65,156] In addition, some anaerobic rods such as *F. nucleatum*, prevotella, and *Campylobacter rectus* can be found in persistent infections.[160]

E. faecalis is the most frequently isolated bacteria from persistent periapical periodontitis[3,22,33,34,37,50,64,65,70,94,99,106,111,127,136,156,162,175,178,180,208] and has many virulence factors.[33,94,99,121,127,136,175,208] *E. faecalis* is a gram-positive, catalase-negative, facultative anaerobic bacterium. Most strains are non-hemolytic and non-motile.Numerous virulence

factors have been identified with *E. faecalis* such as aggregation substance (AS), gelatinase, cytolysin toxin, extracellular superoxide production, and capsular polysaccharides.[127] Additionally, it has been suggested that *E. faecalis* may have an additional virulence factor allowing it to secrete lipase.[33]

The survival of *E. faecalis* after endodontic therapy has been attributed to various reasons. *E. faecalis* is reported to penetrate deeply within the dentin tubules; thus it is able to resist the current chemo-mechanical model of endodontic therapy.[66,158] Additionally, *E. faecalis* is resistant to the inter-appointment placement of calcium hydroxide. The antimicrobial effect of calcium hydroxide is related to the high pH that it produces, and *E. faecalis* can withstand this because of a proton pump in its membrane.[127] Moreover, *E. faecalis* is reported to be capable of forming biofilms in root canals, which can greatly enhance its resistance to antimicrobial regimens.[30] As stated previously, findings of a systematic review by Estrela et al.[36] in 2008 show that NaOCl as well as CHX were ineffective in eliminating *E. faecalis* when evaluated by either PCR or culture techniques.

Prevotella intermedia is a black pigmented gram-negative anaerobic rod commonly found in primary endodontic infections and is also often associated with acute apical abscesses.[156,160] It has several virulence factors and recently has been shown to withstand oxidative stress, allowing it to survive in a higher oxygen environment.[144] It has also been suggested that *P. intermedia* might increase the activity of degradative host-derived enzymes such as esterase, esterase-lipase, acid-phosphatase and alpha-fucosidase when present in active periodontal infection sites.[97] Additionally, *P. intermedia* has been shown to degrade both natural and synthetic substrates; however,

there is intra-species variability in this virulence factor.[176] While most microorganisms favoring conditions of a primary endodontic infection are eliminated after chemo-mechanical instrumentation, some anaerobic rods, such as *P. intermedia*, are able to persist.[19,49,141]

Pseudomonas aeruginosa is a gram-negative aerobic rod that can be found in secondary endodontic infections.[67,131] *P. aeruginosa* has several virulence factors that play important roles in its pathogenicity. *P. aeruginosa* is known to secrete many extracellular proteins such as lipase, phospholipase, alkaline phosphatase, exotoxin, elastase, and alkaline protease.[88,190] The secretion of extracellular proteins is controlled by quorum sensing, which also plays an important role in the ability of *P. aeruginosa* to form biofilms as well as resist antibiotic therapy.[200] The presence of *P. aeruginosa* is highly suggestive of a secondary infection because it is a bacterial species not commonly found in the oral cavity.[131,156]

Historically, information obtained about microorganisms present in infected root canals has been done with a culturing technique. This technique only gives a glimpse into the scope of an endodontic infection, due to the fact that only 50 percent of oral bacteria are culturable.[137] More recent studies are using polymerase chain reaction (PCR), which amplifies DNA present within the canal space, allowing for detection of unculturable bacteria. Rolph et al.[137] demonstrated that molecular techniques can detect the presence of bacteria in endodontic infections when culture techniques yield a negative result and can be used to identify a wider range of endodontic infection related bacteria including the presence of previously unidentified or unculturable bacteria. Findings from molecular techniques are leading to a paradigm shift in our understanding of endodontic

infection. For instance, studies have shown that while *E. faecalis* is very prevalent in persistent infections, multiple other species also play significant roles.[23] Molecular methods such as PCR and pyrosequencing are faster and have increased sensitivity and accuracy when compared with culturing techniques.[157] The use of molecular techniques for the evaluation of endodontic pathogens is ever expanding our knowledge base on the bacteria involved in the pathogenesis of periradicular diseases.[161]

OBTURATION MATERIALS

Proper obturation of the root canal system is a fundamental step in the sealing of the canals to prevent contamination of the periodontal tissues and subsequent periapical pathosis.[148] Coolidge[25] explained that the primary purpose of root canal obturation is to seal the apical foramen as well as obliterate the canal. The root canal system must be obturated with a material capable of completely preventing communication between the oral cavity and the periapical tissue.[83] To achieve this, many endodontic filling materials and techniques have been advocated and explored throughout the history of endodontics.

Root canal filling materials have been classified as solid-core filling materials, semisolid core, and paste filling materials. An evolution of root canal obturation materials has occurred over time, but none to date has demonstrated all 11 requirements for an ideal root canal filling as described by Grossman.[54] He described the ideal root canal filling to be: 1) easily manipulated and provides ample working time; 2) dimensionally stable with no shrinkage once inserted; 3) able to seal the canal laterally and apically, conforming to its complex internal anatomy; 4) nonirritating to the periapical tissues; 5) impervious to moisture and nonporous; 6) unaffected by tissue fluids-no corrosion or oxidization; 7) able to inhibit bacterial growth; 8) radiopaque and easily discernible

radiographically; 9) non-staining to tooth structure; 10) sterile; 11) easily removed from the canal if necessary.

These criteria have guided the development of obturation materials, which have ranged from paste filling materials, semi-solid core filling materials to solid-core filling materials.[76] Silver points are an example of solid core filling materials and are fabricated to the same size as the last file used to prepare the canal. The main advantages were they could be easily inserted and length control was easily obtained. Unfortunately, they did not seal wall laterally or apically due to their lack of plasticity. Leakage of silver points allowed for corrosion, leading to cytotoxic products such as silver sulfides.[151] These disadvantages led to the decline in the use of silver points.

Paste-type filling materials were thought to be a good alternative to semi-solid filling materials because of their potential for excellent canal adaptation. Research has shown that pastes tend to have voids throughout the fill, and the majority leak more than laterally condensed gutta-percha.[172] The most popular paste system was N2 or Sargenti paste. This paste contained paraformaldehyde and was shown to cause severe and permanent toxic effects on periradicular tissues.[145,152] The disadvantages of paste type filling materials outweigh any advantages, thus paste materials, specifically N2, are now considered below the standard of care.[83]

The semi-solid core filling material is currently considered as the closest in replicating Grossman's original criteria for an obturation material.[83] Currently, the most commonly used semi-solid obturation material is gutta-percha. First was used in dentistry in the late 1800s, gutta-percha is currently the gold standard of core obturation materials.[52] Natural pure gutta-percha comes from the dried juice of the Taban tree,

Isonandra percha. 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.[68] In 1942 Bunn discovered that the crystalline phase of gutta-percha existed in two forms: alpha and beta phases.[52] Differences were noted in the thermal and volumetric properties of the phases; however, no differences were observed in mechanical properties.[52] The alpha form of gutta-percha is the natural tree product, whereas the beta form is the processed product used for root canal fillings. When heated, gutta-percha undergoes phase transitions. Heating of the beta phase to 56˚C causes the crystalline structure to change to an amorphous melt after transitioning through the alpha phase. During transformation and rapid cooling back to the beta phase, gutta-percha undergoes significant shrinkage; thus compaction is imperative during obturation.[52] Manufactured gutta-percha cones commercially available are slightly varied in composition, depending on the manufacturer. Generally, gutta-percha cones consist of: 20-percent gutta-percha (matrix), 66-percent zinc oxide (filler), 11-percent heavy metal sulfates (radiopacifier), and 3-percent waxes or resin (plasticizer).[39]

The large proportion of zinc oxide in dental gutta-percha ensures not only radio-opacity, but more importantly, its antimicrobial properties.[108,109] Soderberg et al.[169] demonstrated in an *in-vitro* study that gram-positive bacteria were susceptible to zinc oxide; however, gram-negative aerobic bacteria and streptococci were usually not inhibited. Conversely, Leonardo et al.[89] and Pupo et al.[129] found that zinc oxide inhibited bacterial growth of both gram-positive and gram-negative bacteria. Further support of the antimicrobial properties of gutta-percha was provided by Moorer and Genet[108] who

showed that gutta-percha cones act as a reservoir for zinc oxide to be leached from the material over time, and this leaching can inhibit bacterial growth. Thus, gutta-percha does not support bacterial growth.

Additional advantages to gutta-percha are that it is relatively biocompatible and well-tolerated by periapical tissues. Studies show that after subcutaneous implantation of gutta-percha, it is normally surrounded by a well-defined capsule rich in cells, with few macrophages present.[16,165] On the contrary, when gutta-percha is in the form of very small particles, it can induce an intensive foreign body reaction with massive accumulation of mononucleotide and multinucleated macrophages.[165]

One disadvantage of gutta-percha is that it does not possess any adhesive qualities to bind to dentin. When used alone, gutta-percha is incapable of hermetically sealing a canal. 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.[28] These spaces are present because of physical limitations of the core material 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 obturating material together. Properties of an ideal sealer are[83]:

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 it can be seen radiographically.
4) Very fine powder, so 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, non-irritating to periradicular tissue.

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

There are a few primary categories of endodontic sealers that are currently used in endodontics, the major classes being zinc-oxide eugenol, calcium hydroxide, resin, glass ionomer, and silicones.[28]

In 1964 Rappaport et al.[132] studied 10 common root canal cements and compared their toxicity using an animal model, tissue culture model, and bacteriologic study. AH-26 was not bactericidal or tissue toxic. The most bactericidal cement was zinc oxide and eugenol. According to this study of the cements tested, the AH-26 and zinc oxide eugenol had characteristics that most closely fit the ideal cement.

Zinc oxide-eugenol based materials have long been considered the gold standard of sealers in endodontic therapy. They have several advantages: they absorb if extruded into periradicular tissues, have a slow setting time, and are soluble in common solvents.[83] Similar to gutta-percha, zinc oxide contained within sealers can confer antimicrobial properties. Al-Khatib et al.[4] demonstrated that Grossman's sealer had the greatest overall antibacterial activity when compared with Tubliseal, calciobiotic, Sealapex, Hypocal, Eucapercha, Nogenol and AH26. Some disadvantages to zinc oxide-eugenol sealers are shrinkage on setting, solubility in tissue fluids, and the potential to cause staining of tooth

structure.[83] Additionally, the eugenol in zinc oxide-eugenol sealers has been found to be anti-inflammatory in low concentrations and cytotoxic in high concentrations, if the eugenol contacts tissue.[98]

More recently, resin polymer sealers are available, which include AH26, AH Plus, Diaket and Epiphany. The AH series has been considered the most successful of the traditional resin-based sealers.[117] AH-26 was a slow-setting epoxy resin that is no longer used due to a setting byproduct of formaldehyde.[171] AH Plus is a modified formulation that lacks this toxic byproduct. Benefits of AH Plus include lower solubility than zinc-oxide-eugenol and calcium hydroxide sealers, as well as adequate working time and flow rate.[101] In 2011 Balguerie et al.[9] tested five different kinds of sealer and found that AH Plus had the most optimal tubular penetration and adaptation to the root canal wall of the sealers tested. Resin-based root canal sealers have been found to be more effective in sealing root canals than the zinc oxide-eugenol based sealers.[2]

In the past decade, a new core material, Resilon, has been introduced that is challenging gutta-percha. Resilon is a thermoplastic resin composite specifically designed as a bondable obturating core material. It is used with a dual-cured Bis GMA resin sealer (Epiphany or RealSeal/SE) and self-etching primer. This combination was a new approach to sealing the prepared root canal system. Traditional sealers do not bond to the obturating material or to the canal dentin, and thus there are gaps that could allow microbial leakage into the canal system. Resilon was developed to attempt the formation of a “monoblock” consisting of resin sealer with resin tags that enter into and bond to the dentinal tubule, and to the dentin on the canal wall, as well as bonding adhesively to the

core material.[154] This 'monoblock' type of obturation has led to claims of improved seal, higher fracture resistance, and overall greater clinical success in endodontics.

Resilon is a polymer blend of polycaprolactone, diamethacrylates, mineral fillers and bioactive glass. The filler content within Resilon is considered to be approximately 65 percent by weight.[41] The handling characteristics of Resilon are similar to gutta-percha, such as radio-opacity, retrievability, dissolution with conventional solvents, multiple ISO sizes for master and accessory cones, and seal of the root canal system. The same obturation techniques used for gutta-percha can also be used with Resilon, and although the handling properties are similar, the temperatures required for thermoplastisized techniques are lower for Resilon when compared with gutta-percha.[83]

Resilon is traditionally obturated with RealSeal sealer, formally known as Epiphany, which incorporates the use of self-etching primers.[120] The sealer is a dual-curable resin composite that allows for auto-polymerization within the canals.[182] Together the Resilon and the sealer are supposed to create a monoblock with two interfaces. The first layer is between the sealer and primed dentin and the second is between the sealer and Resilon.[183] In theory, this bond creates a root canal filling that should be impenetrable by microorganisms.[155] The bondability of Resilon to resins is attributed to the incorporation of urethane diamethacrylate resin.[82,183]

Initial leakage studies of Resilon with epiphany sealer showed the Resilon seal to be superior to gutta-percha with conventional sealers.[155,187] Further evaluation of Resilon has brought into question the property of Resilon and a methacrylate resin-based sealer's property to create a monoblock due to polymerization shrinkage stresses that cause debonding and gap formation along the periphery of the root canal filling.[183]

Additionally, the concentration of the polymeric components, polycaprolactone and urethane dimethacrylates are not optimized for ideal adhesion of Resilon to methacrylate resin-based sealers.[183]

Another proposed advantage of Resilon over gutta-percha is the property of the resin bonded filling to resist tooth fracture. Results of an *in-vitro* study done by Teixeira et al.[188] showed that Resilon had a significantly higher resistance to fracture when compared with gutta-percha, regardless of obturation technique used. In 2007 Hammad et al.[69] gave further support for this claim. They found that the force required for vertical root fracture was statistically significantly higher in groups with resin-based obturating materials (Resilon and EndoRez) as opposed to groups obturated with gutta-percha. On the contrary, more recent research is demonstrating that there is no significant difference in fracture resistance between Resilon and gutta-percha with a resin sealer.[85] Additionally, Gesi et al.[43] found that gutta-percha and AH Plus exhibited significantly higher interfacial strength than the Resilon group.

In addition to Resilon's failure to form a complete monoblock, as well as the false claim of increased fractured resistance, the stability of Resilon as a root canal obturation material has also been questioned.[185] The thermoplasticity of Resilon is attributed to the incorporation of polycaprolactone.[81] Polycaprolactone is a synthetic, biodegradable, semi-crystalline aliphatic polyester used in a number of biodegradable and resorbable medical and drug delivery devices.[185] Polycaprolactone is known to be susceptible to both alkaline and enzymatic hydrolysis. Previous studies have shown that the polycaprolactone within Resilon is susceptible to alkaline and enzymatic hydrolysis and have found that Resilon can be degraded by salivary lipases, microbial hydrolysis, and by

microorganisms found in dental sludge.[73,184-186] In addition, lipase has been demonstrated to degrade Resilon.[73] The incorporation of dimethacrylates, mineral, and bioactive glass fillers into the Resilon cones did not prevent degradation. It has been suggested that biodegradation of Resilon may occur after endodontic therapy and compromise the success of the endodontically treated tooth.[73,186] Additionally, it has been shown that Resilon is not impervious to microbial leakage, especially in regards to the apical seal.[182]

Furthermore, residual bacteria thought to be entombed by the obturating material might actually be using Resilon as a biodegradable nutrient source. Tay[184] states that,

> Biodegradability refers to an event which takes place though enzymatic decomposition associated with living organisms. Due to the insolubility of the polymer, constitutional or inducible enzymes are released by microorganisms to depolymerize the biodegradable material before they can utilize the degraded components as carbon sources. It is only during the deprivation of a conventional nutrient source when it is necessary for bacteria to regulate the genes for the transcription of inducible enzymes that are required for the utilization of an alternative carbon source.

The biodegradable material is ultimately converted to water and carbon dioxide under aerobic conditions and/or methane under anaerobic conditions. Due to a significant amount of research disputing the claims that Resilon is a superior obturation material, long term results and clinical trials are warranted to further investigate the benefit of this material as opposed to gutta-percha.

Obturation Techniques

Throughout the history of endodontics several obturation techniques have been developed, such as cold lateral condensation; warm lateral condensation; warm vertical condensation; single-cone methods; injection gutta-percha techniques; paste-only

techniques; thermo-mechanical compaction, and core-carrier technique.[83] Regardless of technique used, Schaeffer et al.[147] showed in a systematic review that better success rate is achieved when treatment includes obturation short of the apex (0 mm-1 mm). Current research underlines the importance of proper biomechanical debridement and then creating a hermetic seal, but debate continues about the best obturation method and material.

Cold lateral condensation is the most common practiced technique.[31] The technique requires a conservative preparation and minimizes the risk of overextension of material into the periapical tissues. In 1992 Glickman and Guttmann[45] outlined several important factors and conditions for cold lateral condensation to be successful, including: 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 vertical and lateral; 3) Placement of the spreader to the appropriate depth without touching the canal walls ensures 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 tugback, and 5) Placement of the spreader should be within 1 mm of the working length adjacent to the master cone. There are several advantages of cold lateral condensation such as: relative ease of use of the material, apical control of material, conservative preparation, low cost, and predictability. In 1993 Wu and Wesselink[205] claimed cold lateral condensation to be the gold standard in obturation and the standard control in obturation studies.

Smith et al.[167] conducted a five-year retrospective study in 1993 on factors that influence the outcome of root canal therapy. The results of their study indicated that cold

lateral condensation of gutta-percha is a sound method for obturation of the root canal space. The sealing ability of lateral condensation has been confirmed by numerous studies.[96,142] Some disadvantages of this method are that it is time consuming, lack of homogeneity of the gutta-percha, increased number of voids and sealer pools, and less adaptation to canal irregularities.[205] One of the greatest pitfalls of lateral condensation is the possibility of iatrogenic vertical root fracture during compaction.[74,102] Modifications of the cold lateral condensation technique were investigated, such as warming spreaders; heating gutta-percha; activation of finger spreaders in a reciprocating handpiece or ultrasonically, and application of a thermo-mechanical compactor to create frictional heat and advance the material apically.

In 1967 Schilder[149] first described the warm vertical condensation technique using gutta-percha to fill the canal in three dimensions. He believe that obturation of the canal with warm, vertically condensed, gutta-percha offers the advantages of good dimensional stability, high density in the apical portion of the canal, and the ability to obturate accessory canals. Brothman[17] showed that vertical condensation of gutta-percha demonstrates more lateral canals than lateral condensation.

Modifications to the Schilder technique were introduced by Buchanan[18] in 1996 to attempt to provide a faster, more efficient, and more effective method of obturation. He incorporated the System B that delivers a continuous heat source for extended periods of time. This modification is currently known as the continuous wave compaction technique. This technique uses various pluggers that match the taper of the non-standardized gutta-percha cones. The System B is used to deliver heat to sealer-coated cones in the canals. The System B delivers heat at a temperature of 200˚C and is

advanced until it is 5 mm to 7 mm from the apical extent of the canal. The remaining portion of the canal is then backfilled using small increments of flowable gutta-percha and then pluggers to condense the gutta-percha as it cools. The Obtura III is currently the most well-known system used to deliver heat plasticized gutta-percha to the root canal system. It delivers gutta-percha at a temperature between 80˚C and 135˚C. Studies have also found that the levels of heat generated by plasticized gutta-percha do not appear to clinically have any negative effects.[61,62] This technique can be used for both gutta-percha and Resilon.

Ventura and Breschi[196] evaluated the quality of endodontic sealing using various obturation techniques. This study found that the continuous wave compaction technique created an effective apical plug with increased apical sealing and a reduction in voids when compared with Schilder's original technique. In 2007 a systematic review was done by Peng et al.[125] on outcomes of warm gutta-percha versus cold lateral condensation technique and found that warm gutta-percha obturation demonstrated a higher rate of overextension than cold lateral condensation. Other factors evaluated were: postoperative pain prevalence, long-term outcomes, and obturation quality. All factors were found to be similar between the two groups.

EVALUATION OF SAMPLES

Scanning Electron Microscope and Energy-Dispersive Spectrometer

The first reported use of the scanning electron microscope (SEM) to evaluate thick specimens was in 1942. Since then improvements have been made that have improved resolution and analysis of samples. Currently the major use of the SEM is to

obtain topographic images in a magnification range of X10 to X10000. SEM examines specimens by irradiating them with a finely focused electron beam that is swept across the specimen to obtain an image.[46] While the SEM provides detailed imaging of a specimen, it is important to note that certain biases can occur with this evaluation method. For example, when evaluating samples under higher magnifications, smaller and smaller segments of the specimen are visible, and this small sample might not be truly representative of the entire specimen. This can lead to operator bias, meaning that most SEM technicians might have a tendency to select the ideal looking area for what they are trying to observe, thus skewing the results of the study.

In 1968 an energy-dispersive spectrometer (EDS) was added to an electron probe microanalyzer to measure x-rays. This technology has since been coupled with instruments such as the SEM. The EDS system allows a rapid evaluation of the elemental constituents of a sample as well as accurate quantitative analysis. EDS is primarily used to measure only the major elements in a sample, which are greater than 10 percent of the weight of the sample.[46] Multiple studies have used SEM and EDS to evaluate surface characteristics and composition of Resilon and gutta-percha.[51,128]

Profilometry

Profilometry has a wide range of uses in research. It is commonly used for the evaluation of restorative dental materials as well as implant surfaces and the erosion of enamel.[5,150,168] Additionally it has several uses outside of the field of dentistry an example of one being monitoring the response to therapy in a patient with head and neck lymphedema after surgery and radiation treatment.[100] Surface profilometry provides information on surface roughness, commonly known as an R_a or R_q value. This value is

obtained by scanning the surface of a specimen using either a contact or non-contact measuring device. In non-contact profilometry, a white or blue laser light is used; while in contact profilometry, the surface is scanned by a stylus with a diamond or steel tip.[150] In order to obtain maximum sensitivity and accuracy, specimens must be flattened prior to evaluation with profilometry.

STERILIZATION TECHNIQUES

Gutta-percha and Resilon cones as well as obturating pellets are manufactured under aseptic conditions; however, they can become contaminated during handling, by aerosols, and by physical sources during the storage process.[154] The thermoplastic characteristics of these materials eliminate the option of sterilizing them with conventional techniques. Conventional techniques such as autoclaving and dry heat would cause alterations in the structure of gutta-percha and Resilon, thus causing subsequent dimensional changes, which could result in higher failure rates due to unstable obturating materials.[47] In this experiment, it was important to choose a correct sterilization technique that would prevent alterations to the structure of Resilon and gutta-percha, because changes in the surface and structure were being evaluated. While steam autoclave and dry heat sterilization methods are the most common methods to sterilize dental materials, additional methods such as ultraviolet radiation and ethylene oxide are also used.

Ultraviolet (UV) irradiation has a well known antimicrobial efficacy and has traditionally been used to disinfect water, air, and surface disinfection. The distance from the UV source is relevant, and variations in this can lead to inconsistent antimicrobial efficacy.[199] The most effective wavelength for UV sterilization is 254 nm. It is important

to note, however, that spores have been shown to be 10 times to 50 times more resistant than growing cells.[113] When radiation sterilization is used, the most critical factor is the total dosage of radiation. Typical sterilization using radiation can take as long as 24 hours, which is significantly more time-consuming than the traditional steam autoclave.[203]

Ethylene oxide (EtO) is one of the oldest methods to sterilize extracted teeth.[189] This sterilization procedure is typically done in three steps: 1) Sixteen-hour pre-conditioning under 50 percent to 80 percent relative humidity at 38˚C; 2) EtO gas cycles for 3 hours at 625 ml/L concentration under 40˚C to 50˚C, which is effective for killing all microorganisms including spores; 3) A minimal aeration period of 72 hours at 40˚C is required due to toxicity of ethylene oxide to human tissues.[189] This method of sterilization is time consuming, but it is beneficial in that it does not alter the material it sterilizes.[123] To date, studies have conflicting results on the ability to kill endospores using this technique.[189]

MATERIALS AND METHODS

The proposed experimental model aimed to simulate the microbial-infected, Resilon-obturated root canal space. The susceptibility of Resilon and gutta-percha to degradation by three typical root canal microbiota was evaluated.

PREPARATION OF RESILON AND GUTTA-PERCHA SAMPLES

Standard Resilon and gutta-percha pellets were used. These pellets were sliced with a sterile #15 blade into 3-mm thick slices and 4 mm in diameter (Figure 1). Pellets were then mounted onto a Streuer Bakelite polishing disc with heated sticky wax (Figure 2) and finished with 1200-, 2400-, and 4000-grit silicon carbide paper (SiC) under water refrigeration (Figure 3). All samples were measured by profilometry for surface roughness before and after the experiment (Figure 4). Five random samples of each material were analyzed by SEM and elemental analysis to obtain a baseline value prior to the experiment.

Sterilization of Samples

All 40-ml vials were autoclaved prior to placement of discs containing obturating material. Discs with obturating material were placed under UV light for 24 hours (Figure 5). After UV light exposure, discs were removed with sterile gloves and transferred to vials. Strict sterile technique was followed for adding broth to samples (Figure 6).

Bacterial Cultures

E. faecalis (ATCC) and *P. aeruginosa* PA14 (mutant 3A8; kindly provided by Dr. Gregory Anderson, IUPUI) were grown in 40-ml plastic vial containers (2 vials for each bacterium) with tryptic soy broth (TSB; Difco Laboratories, Inc., Detroit, MI). *P. intermedia* (ATCC 25611) was grown in 40- ml plastic containers with brain heart infusion + yeast extract and vitamin K in vials containing samples of the obturating material (either Resilon or gutta-percha) mounted on polishing discs (Figure 7). For each bacterial culture there were two vials, one of which contained Resilon samples, and the other contained gutta-percha samples. Two vials were used for a negative control, both contained only TSB. In the control vials one contained Resilon and the other contained gutta-percha. Twenty-five ml of TSB was placed in each vial (1 disc/vial, 8 vials total). Each vial contained one polishing wheel with eight discs mounted on it, and each vial was inoculated with one of the following: *P. intermedia*, *E. faecalis* or *P. aeruginosa* (positive control). Two vials were used for each bacterium and the controls (8 vials total). The negative control was not inoculated with bacteria. A total of six vials contained 25 ml of TSB each and two vials (*P. intermedia* samples) contained 23.75 ml of BHI + YE and 1.25 ml of vitamin K each and were incubated under aerobic conditions at $37^{0}C$ with 5-percent CO_2. Prior to inoculation of the vials, each microorganism was grown in its respective broths overnight at $37^{0}C$ in aerobic conditions (Figure 8). A micropipette was used to inoculate each vial with 250 µl of an overnight culture of each bacterium with the exception of the negative control (Figure 9). The negative control consisted of only 25 ml of TSB (1 gutta-percha and 1 Resilon). *P. aeruginosa* served as the positive control due to its known secretion of lipase.[194] The vials were then incubated at a temperature of

37^0C under aerobic (5-percent CO_2) conditions that enabled the bacterial growth. Every 3 to 4 days a 10-ml pipette was used to extract all the liquid and 25 ml of fresh sterile TSB/BHE+YE vit K was added to each vial to provide nutrients, which ensured the viability of microbial cells (Figure 10).

Experimental Groups

Four experimental groups were used in addition to a positive and negative control. The experimental groups were as follows: Group A: Obtura gutta-percha (Spartan, Fenton, MO) with *P. intermedia*, Group B: Resilon (Resilon Research LLC, Madison, CT) with *P. intermedia* Group C: Obtura gutta-percha with *E. faecalis*, Group D: Resilon with *E. faecalis*; Group E positive control with *P. aeruginosa* using gutta-percha; Group F positive control with *P. aeruginosa* using Resilon; Group G negative control with Resilon, Group H negative control with gutta-percha. Each vial contained one polishing wheel with eight discs that had been inoculated with one of the aforementioned bacteria and were incubated for a total of one month. Groups E and F, the positive control, consisted of two TSB vials that each contained a polishing wheel with eight disc samples (one with Resilon and one with gutta-percha) and 250 µl of *P. aeruginosa,* which secretes lipase,[194] known to cause degradation of Resilon.[73] Group G and H consisted of two vials containing only TSB, one with on with gutta-percha and one with Resilon, in order to serve as a negative control. The negative control was used to confirm if degradation occurs without the presence of bacteria.

In the course of the experiment the negative control became contaminated. To investigate the contamination, all vials were plated to ensure that A and B, C and D, and E and F had uniform cultures within each bacterial sample, and G and H were cultured to

ensure that contaminates did not match the morphology of bacteria from groups A to F (Figure 11). Additionally, the plates were compared to plates containing the respective bacteria to ensure similar colony morphology, shape and color. Two additional samples were prepared for a new negative control, to ensure that the broth in and of itself does not alter the structure of gutta-percha or Resilon. The new negative controls were as follows: Group I negative control + 2 ml of gentamicin, 2 ml of cleocin, with gutta-percha and Group J was the negative control + 2 ml of gentamicin, 2 ml of cleocin with Resilon. After one month the discs were removed from each vial and rinsed with 70-percent isopropyl alcohol. All samples were then examined for degradation using profilometry, SEM and elemental analysis.

ANALYSIS OF SAMPLES

Five random samples from each material were randomly selected to obtain a baseline value for SEM and elemental analysis. It was important to obtain a baseline to ensure that at the baseline there is no significant difference between samples. All samples were subjected to profilometry before and after the experiment to obtain qualitative data regarding surface roughness.

Profilometry

All samples were measured by profilometry for surface roughness before and after the experiment (Figure 4). An area of 1 mm x1 mm in the center of the specimen's polished surface was analyzed by optical profilometry. The following parameters were used: step size of 0.002 mm and 0.01 mm in the X and Y directions, respectively; sample rate of 100Hz; and repetition of 1. Scans were performed with sensor S5/03 (Proscan,

Scantron). The mean surface roughness (X and Y directions) of the scanned area was calculated by the dedicated software (Proscan, Scantron) and expressed as R_a.

SEM

Discs were mounted on 12-mm aluminum stubs with carbon adhesive tape and examined using a scanning electron microscope (JEOL model 5310LV, JEOL Ltd., Peabody, MA) equipped with an EDAX EDS detector (EDAX/Amatek, Berwyn, PA) (Figure 12). Samples were then placed in a vacuum desiccator for two weeks prior to SEM and EDS analysis (Figure 13). Surface topographical features were examined at a low accelerating voltage of 10KeV. EDS analysis was done at an accelerating voltage of 10KeV. The weight and atomic percentages of specific elements were evaluated for possible changes over time. The elements that were analyzed for Resilon were bismuth, zinc, oxygen and carbon; and for gutta-percha the elements were carbon, oxygen and zinc. If large changes were observed in these peaks, specifically if the peaks of these elements decreased, then this finding was correlated with degradation of the respective materials.

SEM samples were analyzed for holes in the surface, on a scale from 1 to 4. Samples were evaluated at X200 magnification. The disc was centered on the SEM screen at a low magnification of X50 so the entire disc was visualized, and then magnification was then increased to X200, so all samples included the center dimension of the disc; the exact dimension was 0.925 mm x 0.725 mm. Within this area, a loss of homogeneity was evaluated. Baseline values were obtained from the five randomly selected gutta-percha samples and the five randomly selected Resilon samples for gutta-percha and Resilon baselines, respectively. Baseline samples were taken to demonstrate

the appearance of undisturbed finished sample, which was considered a homogeneous surface free of surface irregularities or "holes" (score of 1). Mild degradation (score of 2) was present if 25 percent of the area visualized had holes (surface irregularities) visualized by SEM. Moderate degradation (score of 3) was considered to mean 50 percent to 75 percent of the area had surface irregularities for an observed area. Severe degradation (score of 4) was found if 75 percent or greater of the area showed destruction of the smooth surface, and the sample as an example of this was the completely degraded Resilon sample taken from a clinical patient (Figures 15 and 16).

STATISTICAL ANALYSIS

Kappa and weighted kappa statistics were calculated to assess inter-rater agreement for the degradation scores. Kappa measures exact agreement, while weighted kappa uses the ordered nature of the categories to "weight" the level of disagreement. Using the maximum degradation score from the two raters, Mantel-Haenszel chi-square tests were used to compare the groups for differences in degradation scores. Two-way analyses of variance were used to examine the effects of material and bacteria on the ranks of the R_a, wt%, and atomic% measurements.

RESULTS

The overall results of this project were inconclusive. Some significant relationships were found, however they did not confirm or contradict the null hypothesis. The findings are divided into four sections; degradation, comparison of materials, gutta-percha comparisons, and Resilon comparisons. The significant relationships that were found are listed below. Additionally Table I to III provide further information about all results regardless of significance.

DEGRADATION

Inter-rater agreement for the degradation scores was poor (kappa = 0.18; weighted kappa = 0.39). For comparisons among the groups for differences in degradation scores, when the scores were collapsed into “any degradation” against “no degradation,” the gutta-percha contaminated control had significantly less degradation than Resilon with *P. intermedia*, Resilon with *E. faecalis*, gutta-percha with *P. aeruginosa*, Resilon with *P. aeruginosa*, Resilon contaminated control, and Resilon negative control. When the full degradation scores were used additional differences were found: Resilon negative control and Resilon *P. aeruginosa* had significantly more degradation than gutta-percha with *P. intermedia*, gutta-percha with *E. faecalis*, gutta-percha with *P. aeruginosa*, and gutta-percha negative control. Resilon with *E. faecalis* and the Resilon-contaminated control had significantly more degradation than gutta-percha with *P. intermedia*, gutta-percha with *E. faecalis*, and gutta-percha with *P. aeruginosa*; and Resilon with *P. intermedia* had significantly more degradation than gutta-percha with *E. faecalis*.

MATERIAL COMPARISONS FOR R_a AND ELEMENTAL ANALYSIS

Gutta-percha had a significantly higher R_a baseline than Resilon for *E. faecalis* and a significantly lower R_a baseline than Resilon for *P. aeruginosa* and contaminated control. Gutta-percha had a significantly lower R_a Post than Resilon for contaminated control. Gutta-percha had a significantly less change in R_a than Resilon for contaminated control. Gutta-percha had significantly less absolute change in R_a than Resilon for *P. intermedia* and contaminated control. Gutta-percha had significantly higher wt% C K and wt% O K than Resilon. Gutta-percha had significantly lower wt% ZnL than Resilon for *E. faecalis* and *P. aeruginosa* and significantly higher wt% ZnL than Resilon for *P. intermedia* and contaminated control. Gutta-percha had significantly higher atomic% C K than Resilon for *P. intermedia* and contaminated control. Gutta-percha had significantly higher atomic% O K than Resilon for *P. aeruginosa* and significantly lower atomic% O K than Resilon for contaminated control. Gutta-percha had significantly lower atomic% ZnL than Resilon for *E. faecalis* and *P. aeruginosa* and significantly lower atomic% ZnL than Resilon for the contaminated control.

BACTERIAL COMPARISONS FOR R_a AND ELEMENTAL ANALYSIS FOR GUTTA-PERCHA

E. faecalis and *P. intermedia* had significantly higher R_a baselines than *P. aeruginosa* and the contaminated control. *E. faecalis* and *P. aeruginosa* had a significantly higher R_a Post than the contaminated control, and *P. aeruginosa* had a significantly higher R_a Post than *P. intermedia*. *P. aeruginosa* had significantly more increase in R_a and more absolute change in R_a than *E. faecalis*, *P. intermedia*, and the contaminated control.

E. faecalis and *P. aeruginosa* had a significantly lower wt% C K than *P. intermedia*, contaminated control, and negative control, and the contaminated control had significantly lower wt% C K than negative control. *P. aeruginosa* had significantly higher wt% O K than *P. intermedia*, the contaminated control, and the negative control; *E. faecalis* had a significantly higher wt% O K than *P. intermedia* and the negative control; and the contaminated control had significantly higher wt% O K than *P. intermedia*. *E. faecalis* had significantly higher wt% ZnL than *P. aeruginosa*, *P. intermedia*, the contaminated control, and the negative control; *P. aeruginosa* had significantly higher wt% ZnL than *P. intermedia* and the negative control; *P. intermedia* and the contaminated control had significantly higher wt% ZnL than the negative control; and the contaminated control had significantly higher wt% ZnL than *P. intermedia*. *E. faecalis* and *P. aeruginosa* had significantly lower atomic% C K than *P. intermedia*, the contaminated control, and the negative control, and the contaminated control had significantly lower atomic% C K than *P. intermedia* and the negative control. *P. aeruginosa* had significantly higher atomic% O K than *E. faecalis*, *P. intermedia*, the contaminated control, and the negative control; *E. faecalis* had significantly higher atomic% O K than *P. intermedia*, the contaminated control, and the negative control; and the contaminated control had significantly higher atomic% O K than *P. intermedia* and the negative control. *E. faecalis* had significantly higher atomic% ZnL than *P. aeruginosa*, *P. intermedia*, the contaminated control, and the negative control; *P. aeruginosa* had significantly higher atomic% ZnL than *P. intermedia* and the negative control; *P. intermedia* and the contaminated control had significantly higher atomic%

ZnL than the negative control; and the contaminated control had significantly higher atomic% ZnL than *P. intermedia*.

BACTERIAL COMPARISONS FOR R_a AND ELEMENTAL ANALYSIS FOR RESILON

P. intermedia and the contaminated control had significantly higher R_a baseline than *E. faecalis*, and *P. intermedia* had significantly higher R_a baseline than *P. aeruginosa*. *P. aeruginosa* and the contaminated control had significantly higher R_a Post than *E. faecalis*. *E. faecalis*, *P. aeruginosa*, and the contaminated control had significantly more increase in R_a than *P. intermedia*. *E. faecalis* had significantly less absolute change in R_a than *P. intermedia*.

The negative control had significantly higher wt% C K than *E. faecalis*, *P. aeruginosa*, *P. intermedia*, and the contaminated control; and *E. faecalis* and *P. aeruginosa* had significantly higher wt% C K than *P. intermedia* and the contaminated control. *E. faecalis* and *P. aeruginosa* had significantly higher wt% O K than *P. intermedia*, the contaminated control, and the negative control; and the contaminated control had significantly higher wt% O K than *P. intermedia*. *E. faecalis* had significantly higher wt% ZnL than *P. aeruginosa*, *P. intermedia*, the contaminated control, and the negative control; *P. aeruginosa* had significantly higher wt% ZnL than *P. intermedia*, the contaminated control, and the negative control; and *P. intermedia* and the contaminated control had significantly higher wt% ZnL than negative control. *P. intermedia* and the contaminated control had significantly higher wt% BiM than *E. faecalis*, *P. aeruginosa*, and the negative control. *P. intermedia* and the negative control had significantly higher atomic% C K than *E. faecalis*, *P. aeruginosa*, and the contaminated control. *E. faecalis*,

P. aeruginosa, and the contaminated control had significantly higher atomic% O K than *P. intermedia* and the negative control. *E. faecalis* had significantly higher atomic% ZnL than *P. aeruginosa*, *P. intermedia*, the contaminated control, and the negative control; *P. aeruginosa* had significantly higher atomic% ZnL than *P. intermedia*, the contaminated control, and the negative control; and *P. intermedia* and the contaminated control had significantly higher atomic% ZnL than the negative control. *P. intermedia* and the contaminated control had significantly higher atomic% BiM than *E. faecalis*, *P. aeruginosa*, and the negative control.

FIGURES AND TABLES

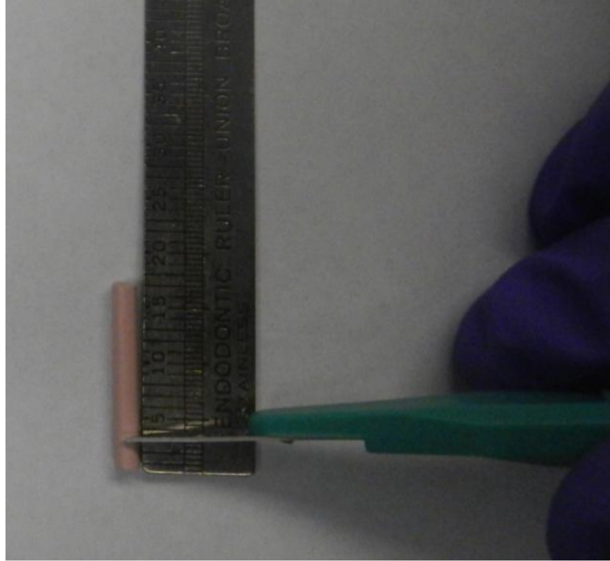

FIGURE 1. Cutting gutta-percha and Resilon pellets with 15-blade scalpel.

FIGURE 2. Samples mounted on ceramic discs with sticky wax.

FIGURE 3. Polishing of samples.

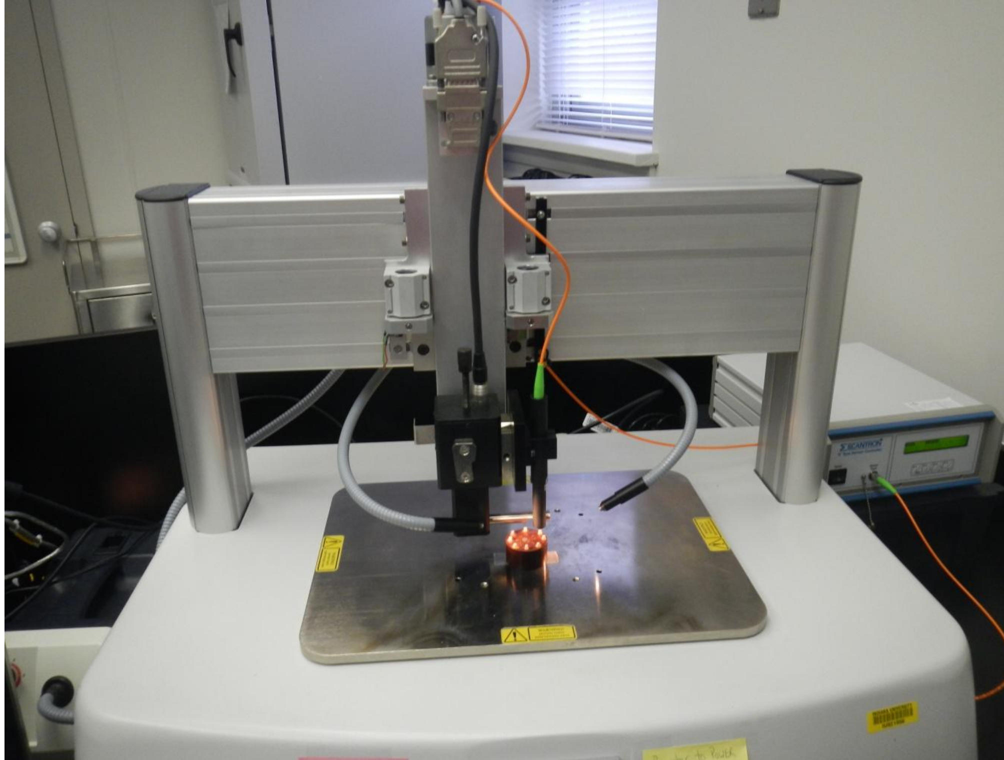

FIGURE 4. Samples measured by profilometry before and after experiment.

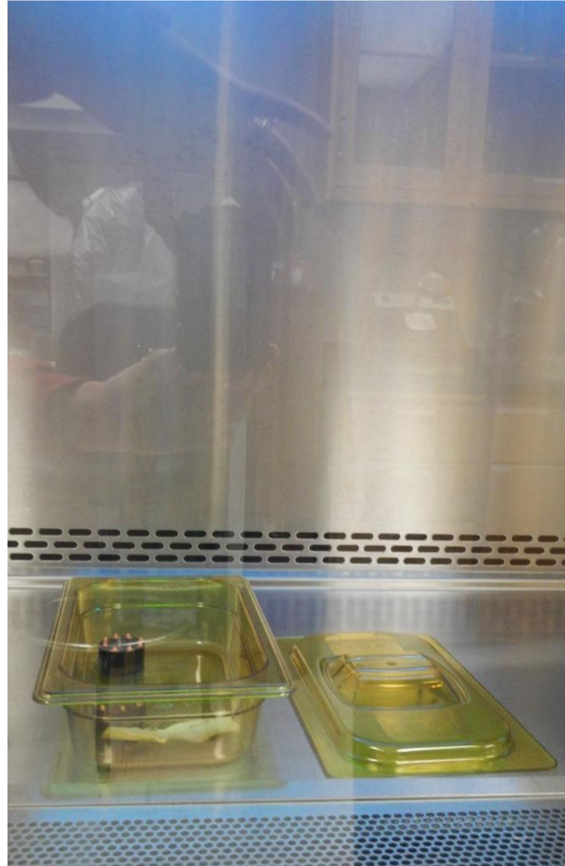

FIGURE 5. Samples sterilized under UV light for 24 hours.

FIGURE 6. Adding broth to vials.

FIGURE 7. Vials with broth.

FIGURE 8. Incubator with samples in incubator.

FIGURE 9. Micropipette used to inoculate samples.

FIGURE 10. Broth removed with 10-ml pipette every 3 to 4 days and fresh broth was added to ensure viability of microbial cells.

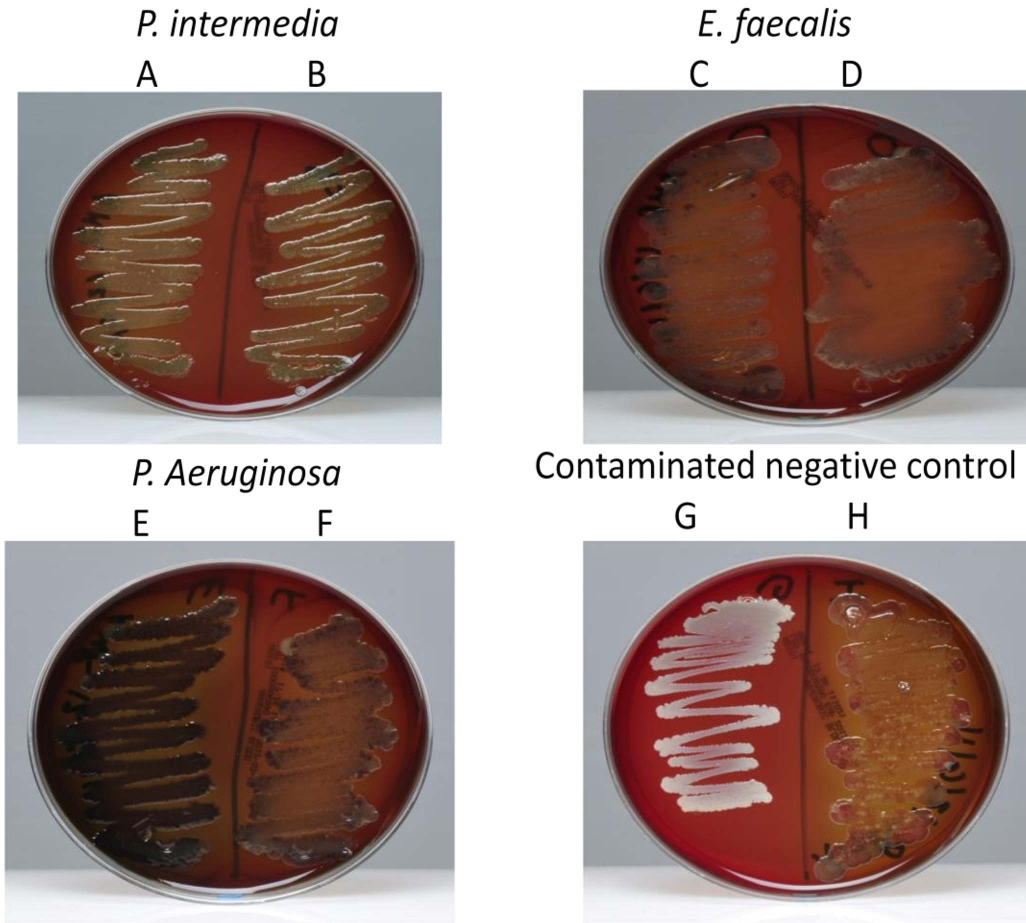

FIGURE 11. Plates of A through H to evaluate uniformity of bacterial species.

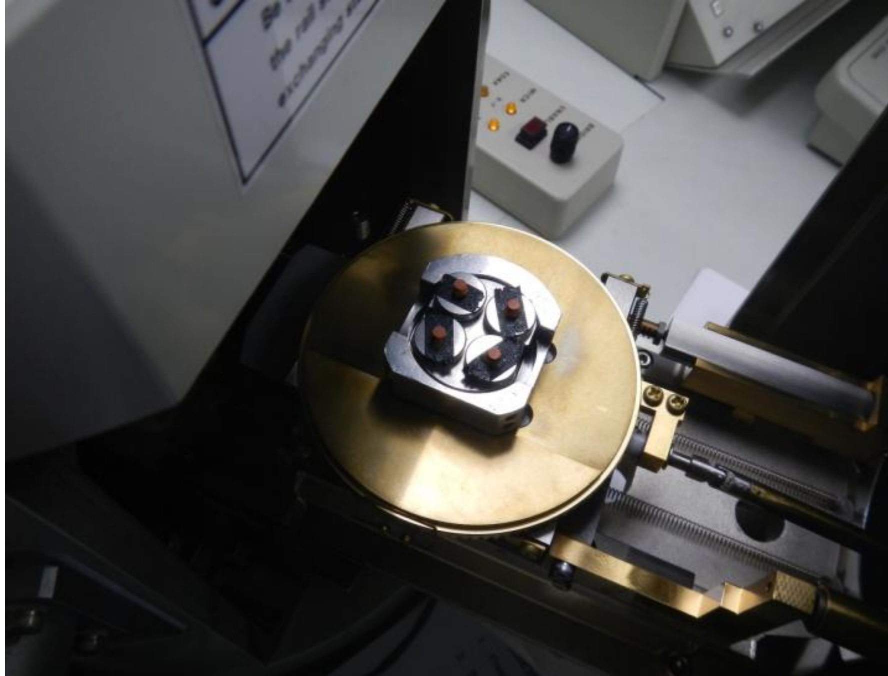

FIGURE 12. Mounted samples.

FIGURE 13. Samples placed in vacuum desiccator for 2 weeks prior to SEM and EDS analysis.

FIGURE 14. Visual comparison of Resilon to gutta-percha following bacterial exposure.

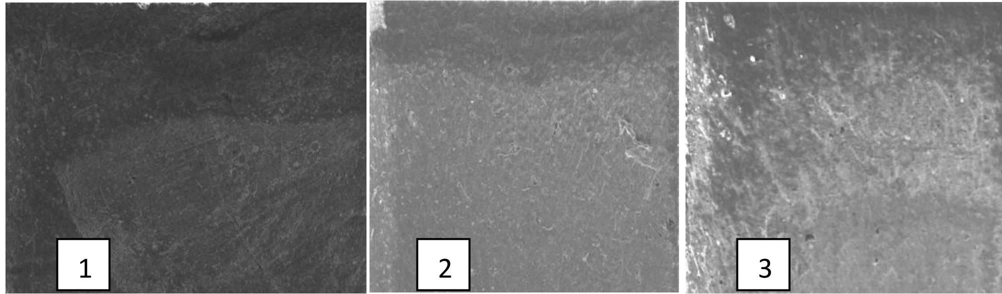

FIGURE 15. Grading (1-4 scale) of gutta-percha samples from left to right, (no gutta-percha graded 4) for gutta-percha.

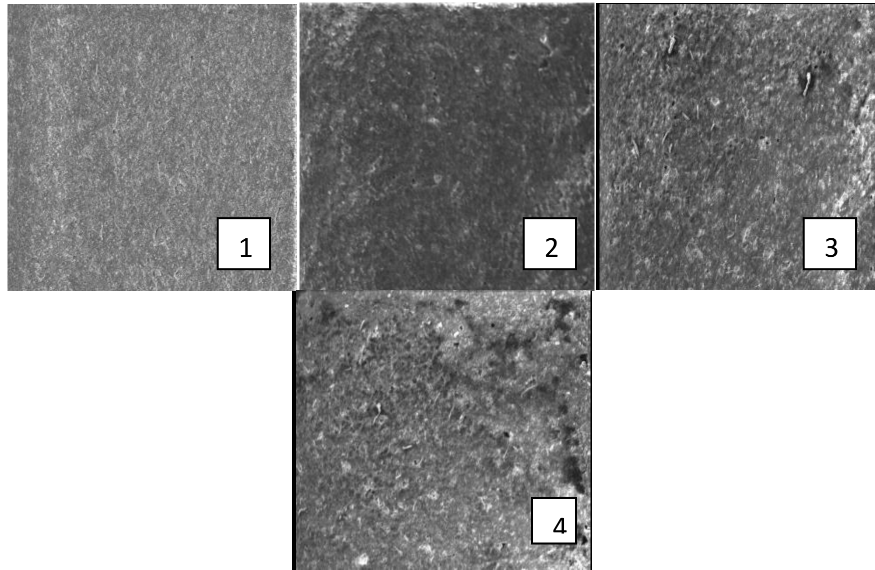

FIGURE 16. Grading (1-4) of Resilon samples from left to right.

TABLE I

Degradation inter-rater agreement, comparison of agreement between observers

		Rater 2					
	Degradation	1	2	3	4	Kappa	Weighted Kappa
Rater 1	1	14 (18%)	22 (28%)	1 (1%)	0 (0%)	0.18	0.39
	2	2 (3%)	12 (15%)	11 (14%)	0 (0%)		
	3	0 (0%)	5 (6%)	7 (9%)	3 (4%)		
	4	0 (0%)	0 (0%)	2 (3%)	1 (1%)		

TABLE II
Degradation scores of 1 to 4 as defined in materials and methods

	Degradation Score			
Group	1	2	3	4
Gutta-percha, *P. intermedia*	3 (38%)	4 (50%)	1 (13%)	0 (0%)
Resilon, *P. intermedia*	1 (13%)	3 (38%)	3 (38%)	1 (13%)
Gutta-percha, *E. faecalis*	3 (38%)	5 (63%)	0 (0%)	0 (0%)
Resilon, *E. faecalis*	0 (0%)	3 (38%)	4 (50%)	1 (13%)
Gutta-percha, *P. aeruginosa*	0 (0%)	8 (100%)	0 (0%)	0 (0%)
Resilon, *P. aeruginosa*	0 (0%)	1 (13%)	5 (63%)	2 (25%)
Resilon, contaminated control	0 (0%)	4 (50%)	4 (50%)	0 (0%)
Gutta-percha, contaminated control	5 (63%)	3 (38%)	0 (0%)	0 (0%)
Resilon, negative control	0 (0%)	2 (25%)	4 (50%)	2 (25%)
Gutta-percha, negative control	2 (25%)	3 (38%)	3 (38%)	0 (0%)
	Degradation			
	No	Yes		
Gutta-percha, *P. intermedia*	3 (38%)	5 (63%)		
Resilon, *P. intermedia*	1 (13%)	7 (88%)		
Gutta-percha, *E. faecalis*	3 (38%)	5 (63%)		
Resilon, *E. faecalis*	0 (0%)	8 (100%)		
Gutta-percha, *P. aeruginosa*	0 (0%)	8 (100%)		
Resilon, *P. aeruginosa*	0 (0%)	8 (100%)		
Resilon, contaminated control	0 (0%)	8 (100%)		
Gutta-percha, contaminated control	5 (63%)	3 (38%)		
Resilon, negative control	0 (0%)	8 (100%)		
Gutta-percha, negative control	2 (25%)	6 (75%)		

TABLE III

Summary of results including roughness comparisons and EDS element percentages comparisons between samples and among groups with the same sample

Measurement	Material	Bacteria	N	Mean	SD	SE	Min	Max
R_a Baseline	Gutta-percha	*E. faecalis*	8	0.88	0.78	0.28	0.38	2.66
		P. aeruginosa	8	0.39	0.08	0.03	0.33	0.56
		P. intermedia	8	0.80	0.77	0.27	0.43	2.69
		contaminated control	8	0.33	0.03	0.01	0.27	0.38
		negative control	0					
	Resilon	*E. faecalis*	8	0.43	0.04	0.01	0.39	0.51
		P. aeruginosa	8	0.47	0.06	0.02	0.42	0.60
		P. intermedia	8	1.65	1.03	0.36	0.43	2.82
		contaminated control	8	0.56	0.11	0.04	0.45	0.73
		negative control	0					
R_a Post	Gutta-percha	*E. faecalis*	8	1.06	0.66	0.23	0.63	2.65
		P. aeruginosa	8	1.05	0.36	0.13	0.76	1.65
		P. intermedia	8	2.36	4.46	1.58	0.55	13.37
		contaminated control	8	0.60	0.13	0.04	0.43	0.81
		negative control	0					
	Resilon	*E. faecalis*	8	0.78	0.08	0.03	0.65	0.92
		P. aeruginosa	8	0.89	0.10	0.04	0.78	1.06
		P. intermedia	8	0.86	0.08	0.03	0.80	1.03
		contaminated control	8	1.00	0.14	0.05	0.80	1.17
		negative control	0					

(continued)

TABLE III (cont.)

Summary of results including roughness comparisons and EDS element percentages comparisons between samples and among groups with the same sample

Measurement	Material	Bacteria	N	Mean	SD	SE	Min	Max
R_a Post-baseline Diff	Gutta-percha	*E. faecalis*	8	0.18	0.28	0.10	-0.39	0.47
		P. aeruginosa	8	0.67	0.30	0.10	0.41	1.21
		P. intermedia	8	1.56	3.70	1.31	-0.08	10.67
		contaminated control	8	0.27	0.12	0.04	0.12	0.47
		negative control	0					
	Resilon	*E. faecalis*	8	0.35	0.09	0.03	0.23	0.51
		P. aeruginosa	8	0.42	0.07	0.03	0.34	0.51
		P. intermedia	8	-0.79	1.00	0.35	-2.00	0.39
		contaminated control	8	0.44	0.16	0.06	0.23	0.70
		negative control	0					
R_a Abs (Post-baseline)	Gutta-percha	*E. faecalis*	8	0.28	0.16	0.06	0.01	0.47
		P. aeruginosa	8	0.67	0.30	0.10	0.41	1.21
		P. intermedia	8	1.58	3.69	1.31	0.04	10.67
		contaminated control	8	0.27	0.12	0.04	0.12	0.47
		negative control	0					
	Resilon	*E. faecalis*	8	0.35	0.09	0.03	0.23	0.51
		P. aeruginosa	8	0.42	0.07	0.03	0.34	0.51
		P. intermedia	8	1.07	0.64	0.23	0.34	2.00

(Continued)

TABLE III (cont.)

Summary of results including roughness comparisons and EDS element percentages comparisons between samples and among groups with the same sample

Measurement	Material	Bacteria	N	Mean	SD	SE	Min	Max
R_a Abs (Post-baseline)	Resilon	contaminated control	8	0.44	0.16	0.06	0.23	0.70
		negative control	0					
Wt% C K	Gutta-percha	*E. faecalis*	8	56.66	1.32	0.47	54.55	58.10
		P. aeruginosa	8	56.88	1.22	0.43	54.70	58.52
		P. intermedia	8	68.36	2.35	0.83	66.60	73.84
		contaminated control	8	62.85	1.73	0.61	60.69	65.46
		negative control	8	72.00	7.62	2.69	60.52	83.97
	Resilon	*E. faecalis*	8	52.51	2.83	1.00	48.07	55.42
		P. aeruginosa	8	52.81	1.89	0.67	49.72	55.14
		P. intermedia	8	45.00	3.17	1.12	42.12	52.48
		contaminated control	8	44.97	5.71	2.02	39.05	54.47
		negative control	7	70.52	18.49	6.99	48.28	100.00
Wt% O K	Gutta-percha	*E. faecalis*	8	34.66	1.32	0.47	32.88	36.39
		P. aeruginosa	8	37.49	1.01	0.36	36.49	39.36
		P. intermedia	8	28.73	2.11	0.74	23.90	30.82
		contaminated control	8	32.37	0.91	0.32	31.15	33.81
		negative control	8	28.01	7.62	2.69	16.03	39.48
	Resilon	*E. faecalis*	8	31.62	2.71	0.96	27.00	33.95
		P. aeruginosa	8	32.01	1.82	0.64	30.30	35.55
		P. intermedia	8	21.56	1.23	0.43	20.15	23.95
	Resilon	contaminated control	8	26.57	6.78	2.40	21.10	37.59
		negative control	7	19.34	11.81	4.46	0.00	32.05

(continued)

TABLE III (cont.)

Summary of results including roughness comparisons and EDS element percentages comparisons between samples and among groups with the same sample

Measurement	Material	Bacteria	N	Mean	SD	SE	Min	Max
Wt% ZnL	Gutta-percha	*E. faecalis*	8	**8.68**	1.27	0.45	6.83	10.34
		P. aeruginosa	8	5.64	0.61	0.22	4.79	6.59
		P. intermedia	8	2.92	0.69	0.24	2.07	3.81
		contaminated control	8	4.78	1.30	0.46	3.28	6.51
		negative control	8	0.00	0.00	0.00	0.00	0.00
	Resilon	*E. faecalis*	8	10.65	0.81	0.28	9.55	11.85
		P. aeruginosa	8	7.86	2.60	0.92	4.49	11.06
		P. intermedia	8	1.48	0.47	0.17	0.85	2.10
		contaminated control	8	2.10	1.99	0.70	0.53	6.46
		negative control	7	0.00	0.00	0.00	0.00	0.00
Wt% BiM	Gutta-percha	*E. faecalis*	0					
		P. aeruginosa	0					
		P. intermedia	0					
		contaminated control	0					
		negative control	0					
	Resilon	*E. faecalis*	8	5.23	5.72	2.02	0.88	15.36
		P. aeruginosa	8	7.33	5.45	1.93	0.00	13.20
		P. intermedia	8	31.96	4.41	1.56	21.68	35.50
		contaminated control	8	26.36	13.72	4.85	2.57	38.69
		negative control	7	10.14	17.74	6.71	0.00	42.17

(continued)

TABLE III (CONT.)

Summary of results including roughness comparisons and EDS element percentages comparisons between samples and among groups with the same sample

Measurement	Material	Bacteria	N	Mean	SD	SE	Min	Max
Atomic% C K	Gutta-percha	*E. faecalis*	8	67.23	1.15	0.41	65.87	68.66
		P. aeruginosa	8	66.09	1.06	0.37	64.10	67.31
		P. intermedia	8	75.55	1.94	0.69	73.83	80.09
		contaminated control	8	71.39	1.14	0.40	70.15	73.17
		negative control	8	77.28	6.62	2.34	67.12	87.46
	Resilon	*E. faecalis*	8	66.91	0.73	0.26	65.73	67.72
		P. aeruginosa	8	67.11	0.98	0.34	65.75	68.13
		P. intermedia	8	71.06	0.92	0.32	70.05	72.83
		contaminated control	8	67.52	2.09	0.74	64.36	70.22
		negative control	7	81.82	9.77	3.69	73.07	100.00
Atomic% O K	Gutta-percha	*E. faecalis*	8	30.87	1.16	0.41	29.31	32.00
		P. aeruginosa	8	32.71	1.01	0.36	31.61	34.62
		P. intermedia	8	23.85	1.90	0.67	19.46	25.65
		contaminated control	8	27.61	0.97	0.34	26.13	28.60
		negative control	8	22.72	6.62	2.34	12.54	32.88
	Resilon	*E. faecalis*	8	30.19	1.07	0.38	28.56	31.56
		P. aeruginosa	8	30.53	0.90	0.32	29.48	32.14
		P. intermedia	8	25.59	0.63	0.22	24.73	26.72
		contaminated control	8	29.49	2.97	1.05	26.21	34.03
		negative control	7	17.23	9.66	3.65	0.00	26.15
Atomic% ZnL	Gutta-percha	*E. faecalis*	8	1.90	0.29	0.10	1.47	2.29
		P. aeruginosa	8	1.20	0.14	0.05	1.01	1.42

(continued)

TABLE III (cont.)

Summary of results including roughness comparisons and EDS element percentages comparisons between samples and among groups with the same sample

Measurement	**Material**	**Bacteria**	**N**	**Mean**	**SD**	**SE**	**Min**	**Max**
Atomic% ZnL	Gutta-percha	*P. intermedia*	8	0.59	0.14	0.05	0.42	0.78
		contaminated control	8	1.00	0.28	0.10	0.67	1.38
		negative control	8	0.00	0.00	0.00	0.00	0.00
	Resilon	*E. faecalis*	8	2.50	0.19	0.07	2.15	2.71
		P. aeruginosa	8	1.82	0.56	0.20	1.08	2.48
		P. intermedia	8	0.43	0.13	0.05	0.25	0.63
		contaminated control	8	0.55	0.44	0.16	0.16	1.43
		negative control	7	0.00	0.00	0.00	0.00	0.00
Atomic% BiM	Gutta-percha	*E. faecalis*	0					
		P. aeruginosa	0					
		P. intermedia	0					
		contaminated control	0					
		negative control	0					
	Resilon	*E. faecalis*	8	0.40	0.46	0.16	0.06	1.24
		P. aeruginosa	8	0.55	0.41	0.15	0.00	0.99
		P. intermedia	8	2.93	0.52	0.19	1.73	3.40
		contaminated control	8	2.45	1.38	0.49	0.18	3.88
		negative control	7	0.95	1.70	0.64	0.00	4.19

DISCUSSION

The aim of this study was to determine if Resilon could be degraded by selected pathogenic bacteria of the infected root canal system, and if this degradation was more severe than with conventional obturating material. *P. intermedia*, *E. faecalis* and *P. aeruginosa* were inoculated into vials containing discs of obturating material (Resilon or gutta-percha) and TSB/BHI + YE vitamin K and were incubated at 37°C under aerobic conditions. The discs were finished, examined by SEM, profilometry, and elemental analysis prior to inoculation to establish a baseline. The discs were then re-examined by these methods one month after inoculation. The results were inconclusive due to various reasons.

During the course of the experiment, the negative control became contaminated so an additional negative control was prepared that had cleocin and gentamicin in the TSB broth. In addition to 24 hours under UV light, these samples were also soaked in 70-percent isopropyl alcohol for two hours prior to the addition of antibiotics and TSB. To evaluate the contamination, all vials were plated on blood agar plates to visualize colony morphology, and to compare this with known plates of *P. intermedia*, *E. faecalis* and *P. aeruginosa* to see if bacteria samples were similar in color and morphology (Figure 11). Additionally, the contaminated negative control was evaluated to ensure that the colonies present on these plates were different from the colonies of known bacterial samples. The additional negative control that contained antibiotics was used to ensure that the broth in and of itself does not alter the obturating materials.

Due to the contamination, it is not possible to assume that any changes in Resilon are due to known endodontic pathogens; however, changes can be attributed to bacteria or their enzymes. As a result of these findings, we concluded that the color of Resilon can be changed by exposure to bacteria. Furthermore, the specific bacteria that caused this color change cannot be identified without doing a PCR analysis of bacteria present in the vials. It is possible that the bacterial strains in A through F were pure strains of the respective bacteria from the deposit of a significant amount of a single species of bacteria at the initial inoculums, and bacteriocins or other inhibitory substances from these bacteria could have eliminated or prevented the growth of any potential contaminants. In the future, if this experiment were to be repeated, bacterial strains should be transformed with a resistant gene to a specific antibiotic to create a broth that would select for the bacteria of interest. Additionally, multiple strains of the same bacteria should be used, because there are significant differences in virulence factors between different strains.[93] This would help to eliminate outside contamination.

Prior to SEM, profilometry, and EDS analysis, all samples were subjected to finishing with 1200-grit, 2400-grit, and 4000-grit silicon carbide paper (SiC) under water refrigeration (Figure 3). When polishing with diamond grit was attempted, the diamond grit polish could not be removed from the samples after the procedure; thus samples were only finished to a 4000-grit silicon carbide paper. Ideally, samples should have been polished prior to profilometry. The smoother the surface prior to the procedure, the more effective profilometry is to detect small changes in the surface R_a value.

In this experiment, the profilometry results varied significantly. The overall results were inconclusive. The baseline values between groups showed a significantly

higher baseline R_a for gutta-percha than Resilon in some groups, while other groups had a significantly lower baseline R_a value for gutta-percha then Resilon. Given these were baseline values, and that there was a significant difference among groups prior to the experiment, it was concluded that the material was not smooth enough from finishing.

The samples were still rough enough to have significant differences between the baseline samples of the same material. Therfore, it is difficult to compare groups because the groups were significantly different before the experiment. Rate of change was used to compare groups to address the significant differences between baseline samples. The only significant difference found in the rate of change between gutta-percha and Resilon was in the *P. intermedia* and contaminated-control groups, which had significantly less change in R_a than the respective Resilon groups. In all other groups, no significant difference was seen when comparing the rate of changes of gutta-percha groups to Resilon groups. Within the gutta-percha groups, *P. aeruginosa* had a significant increase in R_a when compared with *E. faecalis*, *P. intermedia*, and the contaminated negative control; however, *P. aeruginosa* also had a significantly lower baseline value than the other groups of gutta-percha. With regard to the Resilon groups, *E. faecalis*, *P. aeruginosa*, and the contaminated control had significantly more increase in R_a than *P. intermedia*. On the other hand, *E. faecalis* had significantly less absolute change in R_a than *P. intermedia*. These findings are confusing and are attributed to the fact that some post R_a values actually decreased, meaning the sample became smoother following the experiment. Additionally, another major problem with the profilometry data is that the negative control was not recorded due to a broken profilometer. As a result, the data are

insignificant, because we are not able to evaluate the effect the broth might have had on the material.

It is possible the significant variation among groups could be attributed to not only the inability to obtain a polished surface, but that the materials absorbed moisture in the atmosphere resulting in alterations of surface structures. It has been reported that the properties of gutta-percha are affected by different humidities because of the plasticizing effect of gutta-percha, attributable to the insertion of water molecules in the polymer chains.[8] It is possible that this property also allows for small changes in the surface topographical features of gutta-percha. If some samples were dryer than others, these materials might have had a significant difference in R_a. Changes in R_a might be attributed to the dryness of the material rather than the effects of specific bacterial species. This could also explain the few anomalies in the data in which the R_a values of the material actually decreased following the experiment. If profilometry were to be used in a future study, all samples should be placed in a humidifier for at least 24 h prior to evaluation.

Another possible explanation is that irregularities could have been produced by the bacterial exposure, resulting in a biofilm formation over the surface, possibly resulting in a smoother surface as was seen in the SEM studies by Takemura.[181] Isopropyl alcohol was used to rinse materials after the experiment; however, the material could not be mechanically cleaned in any way to avoid changing the surface. If a biofilm was present, it is possible that this alcohol rinse was not able to remove the biofilm, resulting in a smoother surface following the experiment.

While it has been shown in the literature that biofilm formation can occur on the surface of gutta-percha,[181] the only microorganisms ever reported in the literature to have

the ability to degrade gutta-percha are six bacterial strain isolates assigned to the genus Nocardia, which utilize synthetic poly(trans-1,4-isoprene) as sole carbon and energy source for growth.[202] It is important to note that both gutta-percha and Resilon are capable of supporting a biofilm; however, no known endodontic pathogen has ever been reported in the literature to be able to degrade gutta-percha. It is unlikely that the significant increase in the R_a value of the *P. aeruginosa* samples would correlate to any kind of degradation process.

Profilometry is traditionally used in dental materials to evaluate harder surfaces, such as differences in porcelain surfaces before and after polishing, or to evaluate demineralization of tooth structure.[53,104,124] The profilometry results of this experiment were inconclusive; the findings do not support or contradict the null hypothesis. It was determined that profilometry is not an ideal method for evaluation of softer materials such as gutta-percha and Resilon used in this way. If profilometry would be used in a future project, rather than in finishing samples, samples should be sliced as if they were being prepared for TEM evaluation, resulting in a very smooth surface. This is the way that samples are prepared for atomic force microscopy. Previous studies on topographical changes in the surface of Resilon and gutta-percha have successfully used atomic force microscopy to show clear results.[78,191] In a future study, this would be a method recommended to evaluate surface features and changes in the surface of gutta-percha and Resilon.

SEM analysis of samples showed significant inter-sample variation within each group, as well as the presence of a significant amount of artifacts. It was extremely difficult to focus the SEM, because the samples were not completely flat and not coated.

The samples also started to melt when the electron beam was directed at them, causing dark spots in the samples that appeared as a disruption or a loss of homogeneity of the surface. Gutta-percha was significantly affected by the SEM, and even the samples used before exposure to bacteria had significant dark areas that appeared to have a loss of homogeneity. When these spots were seen in the post-experiment samples, they were not counted as a loss of homogeneity. They are a result of the electron beam rather than bacterial degradation. The samples were not coated originally because the gold coating can interfere with EDS data, as well as possibly prevent the penetration of bacteria into the sample. For these reasons, the obturating material samples were not coated.

SEM analysis has been used in previous studies to evaluate topographical changes in Resilon.[47,186] In these studies, samples were coated with gold or gold/palladium prior to SEM analysis, which protected the samples from degradation by the electron beam, and also allowed for the samples to be evaluated at a significantly increased magnification. To evaluate the bacterial degradation of Resilon with SEM analysis, samples would have to be coated after exposure to bacteria. The limitation with this method is that the SEM images taken before the experiment were not used in the actual experiment, and the argument could be made that the “before” samples might not be representative of the samples that were actually used in the experiment. In 2005 Tay[185] examined Resilon discs using FE-SEM and found that an accelerating voltage of 20keV was required to evaluate the subsurface filler particles beneath the surface resin layer. In this experiment, the samples were carbon-coated prior to SEM analysis.

The SEM results were inconclusive. The degradation results from the SEM report that *P. aeruginosa* gutta-percha samples are significantly degraded when compared with

other gutta-percha samples. Additionally, the results concluded that the Resilon negative control was significantly degraded. If the negative control showed significant degradation, all samples should have shown significant degradation, unless the antibiotics were the cause of the degradation; however, no studies have shown Resilon is affected by antibiotics. This method of evaluation was not ideal for these materials.

SEM evaluation has many limitations. A significant limitation is that only a small area of a sample is evaluated, and it is possible that this area is not representative of the entire sample. Additionally, there was poor agreement between observers. The grading criteria of 1 to 4 are subjective, and not an appropriate method to obtain quantitative results. If the observers varied, the score differed by only one number, so that the results were similar; there were no blatant discrepancies, such as one observer having a rating of 1, and another a rating of 4. However, there was still a poor overall agreement.

In this project, if the samples had been coated, the magnification would be higher and artifacts such as dust particles would not be as much of a concern, thus possibly resulting in more comprehensible results. If the magnification was increased, however, multiple pictures would have to be taken of each sample; the smaller the sample size, the less likely it is to be representative of the entire sample. In a future study, SEM samples of these materials would need to be coated to best evaluate surface changes of the samples.

Electron dispersive analysis was used by Tay[185] to identify the elemental compositions of the filler in Resilon and gutta-percha. In his study he found that Resilon had two predominant filler types that could be observed at an accelerating voltage of 20 keV, and that the finer, less electron-dense fillers that formed the bulk of the filled

component consisted predominantly of bismuth oxychloride. The samples were not coated in this study; only an accelerating voltage of 10 keV could be used during EDS analysis. Our EDS results from an accelerating voltage of 10 keV identified the main elements of Resilon as carbon, oxygen, zinc and bismuth. Carbon was seen in all samples, both Resilon and gutta-percha baseline and post experiment. Carbon indicates the presence of organic matter. The cones were not sterile, so that bacteria may have been present on the obturating material at one time. The negative control had a significant carbon presence in both gutta-percha and Resilon samples. The presence of carbon does not indicate live bacteria, but it is important to note that all samples contained organic material. Carbon was not seen in previous EDS data in the literature of Resilon and gutta-percha.[185] Chloride was never identified in our Resilon EDS data. It is unclear why this element was not identified, because its atomic weight is within the 10 keV. There was nothing that suggested that the elemental analysis was not functioning properly, so again, it is unclear as to why expected elements were not identified.

In Tay's[185] research the main constituents of gutta-percha identified by EDS were zinc, titanium and oxygen, and they believed that the titanium might have been a result of contamination. In this present study, elements identified in gutta-percha samples were carbon, oxygen and zinc. Gutta-percha had a significantly higher amount of oxygen and carbon when compared with Resilon samples. It is important to note, however, EDS data is not a quantitative measurement, but a qualitative measurement of the particular spot the electron beam is aimed at. The EDS findings of this study were inconclusive. It was concluded that EDS evaluation of these samples is not an ideal way to evaluate these materials for degradation.

One interesting finding of this study was that when Resilon was exposed to bacteria, the color turned from pink to black (Figure 13). This is consistent with clinical findings reported by local clinicians. Sybron Endo released a statement addressing this issue:

> The black substance is Bismuth Sulfide, the result of fluids from leakage (specifically the proteins in the fluid) reacting with the Bismuth Oxychloride in Resilon material added for radio opacity… It was determined that Bismuth Sulfide is not the cause of failure, rather a result. In the EDS data of this experiment, no sulfide was noted, but bismuth was identified. This finding of lack of sulfide in EDS data cannot be given much significance since expected elements were not identified. In failed cases, there was not an appropriate seal between the sealer, filler, and/or dentinal walls which allowed leakage of bodily fluids into the canal.

In this study, the production of bismuth sulfide was seen in response to exposure to bacteria or possibly bacterial enzymes.

It is interesting to note that one of the reasons Resilon was originally marketed as a better product than gutta-percha was that it was supposed to prevent crown-down leakage. However, this study shows that if the product is exposed to bacteria, it turns black, similar to clinical reports of failing Resilon cases. Therefore, if coronal leakage should occur, Resilon is more likely to leak than gutta-percha (Figure 14). This is an interesting finding of this experiment and should be followed up in future studies that include coated samples evaluated under SEM as well as samples evaluated with atomic force microscopy.

SUMMARY AND CONCLUSIONS

The aim of this study was to determine if Resilon could be degraded by selected pathogenic bacteria of the infected root canal system, and if this degradation was more severe than with conventional obturating material. Previous studies have shown that Resilon has no bactericidal or antimicrobial effect.[15] Additionally, it has been shown that Resilon is susceptible to alkaline and enzymatic hydrolysis as well as degradation by dental sludge.[73,184-186] It has been suggested that Resilon may be susceptible to degradation by microorganisms of the root canal space. To date there are no studies evaluating specific an endodontic pathogen's ability to cause degradation of Resilon.

P. intermedia, *E. faecalis* and *P. aeruginosa* were inoculated into vials containing discs of obturating material (Resilon or gutta-percha) and TSB/BHI + YE vitamin K and were incubated at 37°C under aerobic conditions. The discs were finished, examined by SEM, profilometry, and elemental analysis prior to inoculation to establish a baseline, and were then re-examined by these methods one month after inoculation.

The results were inconclusive due to various reasons. Initially, the negative control failed. The materials are not able to be autoclaved, so UV light was used for disinfection. Plated samples appeared to be uniform in accordance with the expected bacteria; however, this study cannot say that any changes were caused by the specific endodontic pathogens used in this study. Next, profilometry results were inconclusive. There were significant differences in baseline groups of the same sample, showing that finishing was not able to leave all samples uniformly smooth. SEM analysis was inconclusive, due to the sensitivity of the samples to the electron beam and a low

magnification. In addition, EDS analysis was not conclusive, and it was determined that this is not a good method for evaluation of the degradation of these obturating materials. EDS is a quantitative, not a qualitative measurement, and cannot be applied to degradation in the way it was used in this experiment.

In conclusion, the overall findings of this study do not support or contradict the null hypothesis. The results were inconclusive due to a variety of factors. However, one notable finding was that Resilon turned black when exposed to bacteria. Future studies are needed to evaluate the significance of this color change as well as Resilon's susceptibility to degradation by endodontic pathogens. Ultimately, it was concluded that this experimental design is an ineffective way to evaluate degradation of Resilon and gutta-percha.

REFERENCES

1 Abou-Rass M, Piccinino MV. The effectiveness of four clinical irrigation methods on the removal of root canal debris. Oral Surg Oral Med Oral Pathol 1982;54:323-8.

2 Adanir N, Cobankara F, Belli S. Sealing properties of different resin-based root canal sealers. J Biomed Mater Res B Appl Biomater 2006;77:1-4.

3 Adib V, Spratt D, Ng YL, Gulabivala K. Cultivable microbial flora associated with persistent periapical disease and coronal leakage after root canal treatment: a preliminary study. Int Endod J 2004;37:542-51.

4 al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML. The antimicrobial effect of various endodontic sealers. Oral Surg Oral Med Oral Pathol 1990;70:784-90.

5 Al-Radha AS, Dymock D, Younes C, O'Sullivan D. Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. J Dent 2012;40:146-53.

6 Anthony LP, Grossman LT. A brief history of root canal therapy in the United States. J Am Dent Assoc 1945;32:43-50.

7 Arias-Moliz MT, Ferrer-Luque CM, Gonzalez-Rodriguez MP, Valderrama MJ, Baca P, Eradication of Enterococcus faecalis biofilms by cetrimide and chlorhexidine. J Endod 2010;36:87-90.

8 Arvanitoyannis I, Kolokuris I, Robinson C, Blanshard JMV, Synergic action of aging and moisture on native and different grades of commercial gutta-percha (trans-polyisoprene).J Appl Polym Sci 1993;47:1905-14.

9 Balguerie E, van der Sluis L, Vallaeys K, Gurgel-Georgelin M, Diemer F. Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study, J Endod 2011;37:1576-9.

10 Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens.J Endod 1987;13:147-57.

11 Becker GL, Cohen S, Borer R. The sequelae of accidentally injecting sodium hypochlorite beyond the root apex. Report of a case. Oral Surg Oral Med Oral Pathol 1974;38:633-8.

12 Bellizzi R, Cruse WP. A historic review of endodontics, 1689-1963 (Pt 3). J Endod 1980;6:576-80.

13 Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontol Revy 1974;25:347-58.

14 Bergenholtz G. Pathogenic mechanisms in pulpal disease. J Endod 1990;16:98-101.

15 Bodrumlu E, Alacam T. The antimicrobial and antifungal activity of a root canal core material. J Am Dent Assoc 2007;138:1228-32.

16 Bodrumlu E, Muglali M, Sumer M, Guvenc T. The response of subcutaneous connective tissue to a new endodontic filling material. J Biomed Mater Res B Appl Biomater 2008;84:463-7.

17 Brothman P. A comparative study of the vertical and the lateral condensation of gutta-percha. J Endod 1981;7:27-30.

18 Buchanan LS. The continuous wave of obturation technique: centered condensation of warm gutta percha in 12 seconds. Dent Today 1996;15: 60-2, 64-7.

19 Bystrom A, Sundqvist G. 'The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.

20 Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8.

21 Cagidiaco MC, Goracci C, Garcia-Godoy F,Ferrari M, Clinical studies of fiber posts: a literature review. Int J Prosthodont 2008;21:328-36.

22 De Paz L Chavez.Gram-positive organisms in endodontic infections. Endod Topic 2004;9:79-96.

23 Chugal N, Wang JK, Wang R, et al. Molecular characterization of the microbial flora residing at the apical portion of infected root canals of human teeth. J Endod 2011;37:1359-64.

24 Clegg MS, Vertucci F, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod 2006;32:434-7.

25 Coolidge ED. Past and present concepts in endodontics. J Am Dent Assoc 1960, 676-88.

26 Cruse WP, Bellizzi R. A historic review of endodontics, 1689-1963 (Pt 1). J Endod 1980;6:495-9.

27 Curson I. History and endodontics. Dent Pract Dent Rec 1965;15:435-9.

28 Orstavik D. Materials used for root canal obturation: technical, biological and clinical testing. Endod Topic 2005;12:25-38.

29 Davies A. The mode of action of chlorhexidine. J Periodontal Res 1973;Suppl 12: 68-75.

30 Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689-93.

31 Dummer PM. Comparison of undergraduate endodontic teaching programmes in the United Kingdom and in some dental schools in Europe and the United States. Int Endod J 1991;24:169-77.

32 Ehrich DG, Brian Jr JD, Walker WA. Sodium hypochlorite accident: inadvertent injection into the maxillary sinus. J Endod 1993;19:180-2.

33 Elsner HA, Sobottka I, Mack D, Claussen M, Laufs R, Wirth R. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. Eur J Clin Microbiol Infect Dis 2000;19:39-42.

34 Engstrom B. The significance of enterococci in root canal treatment. Odontologisk Revy 1964;15:87-106.

35 Esposito PT, Cunningham CJ. A comparison of canal preparation with nickel-titanium and stainless steel instruments. J Endod 1995;21:173-6.

36 Estrela C, Silva JA, de Alencar AH, Leles CR, Decurcio DA. Efficacy of sodium hypochlorite and chlorhexidine against Enterococcus faecalis--a systematic review. J Appl Oral Sci 2008;16:364-8.

37 Gulabivala K Figdor D. Survival against the odds: microbiology of root canals associated with post-treatment disease. Endod Topic 2011;18:62-77.

38 L. Figini, G. Lodi, F. Gorni, and M. Gagliani, 'Single Versus Multiple Visits for Endodontic Treatment of Permanent Teeth: A Cochrane Systematic Review', J Endod, 34 (2008), 1041-7.

39 Friedman CM, Sandrik JL, Heuer MA, Rapp GW. Composition and mechanical properties of gutta-percha endodontic points. J Dent Res 1975:54:921-5.

40 Fujii R, Saito Y, Tokura Y, Nakagawa KI, Okuda K, Ishihara K. Characterization of bacterial flora in persistent apical periodontitis lesions. Oral Microbiol Immunol 2009;24:502-5.

41 Gatewood RS. Endodontic materials.Dent Clin North Am 2007;51:695-712, vii.

42 Gatot A, Arbelle J, Leiberman A, Yanai-Inbar I. Effects of sodium hypochlorite on soft tissues after its inadvertent injection beyond the root apex. J Endod 1991;17:573-4.

43 Gesi A, Raffaelli O, Goracci C, Pashley DH, Tay FR, Ferrari M. Interfacial strength of Resilon and gutta-percha to intraradicular dentin. J Endod 2005;31: 809-13.

44 Gillen BM, Looney SW, Gu LS, et al. Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment:a systematic review and meta-analysis. J Endod 2011;37:895-902.

45 Glickman GN, and Gutmann JL. Contemporary perspectives on canal obturation. Dent Clin North Am 1992;36:327-41.

46 Goldstein, J, Newbury D, Joy D, et al. Scanning electron microscopy and x-ray microanalysis. 3rd ed. New York: Kluwer Academic/Plenum, 2003.

47 Gomes BP, Berber VB, Montagner F, et al. Residual effects and surface alterations in disinfected gutta-percha and resilon cones. J Endod 2007;33:948-51.

48 Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis.Int Endod J 2001;34:424-8.

49 Gomes BP, Lilley LD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. Int Endod J 1996;29:235-41.

50 Gomes BP, Pinheiro ET, Gade-Neto CR, et al. Microbiological examination of infected dental root canals. Oral Microbiol Immunol 2004;19:71-6.

51 Gomes BP, Berber VP, Montagner F, et al. Residual effects and surface alterations in disinfected gutta-percha and Resilon cones. J Endod 2007;33 948-51.

52 Goodman A, Schilder H, Aldrich W. The thermomechanical properties of gutta-percha. The history and molecular chemistry of gutta-percha. Oral Surg Oral Med Oral Pathol 1974;37:954-61.

53 Gregg T, Mace S, West NX, Addy M. A study in vitro of the abrasive effect of the tongue on enamel and dentine softened by acid erosion. Caries Res 2004;38: 557-60.

54 Grossman L. Endodontics. Vol. 11. Philadelphia: Lea & Febiger, 1988.

55 Grossman L. Endodontics 1776-1976: a bicentennial history against the background of general dentistry. J Am Dent Assoc 1976;93:78-87.

56 Grossman L. Endodontics: a peep into the past and the future. Oral Surg Oral Med Oral Pathol 1974;37:599-608.

57 Grossman L. Endodontics: then and now. Oral Surg Oral Med Oral Pathol 1971;32: 254-9.

58 Grossman L. A personal history of dental practice:1920-1980. J Am Dent Assoc 1981;102:360-5.

59 Grossman L. Rationale of endodontic treatment. Dent Clin North Am 1967:483-90.

60 Patel B, Gulabivala K. Effects of mechanical and chemical procedures on root canal surfaces. Endod Topic 2005;10:103-22.

61 Gutmann JL, Creel DC, Bowles WH. Evaluation of heat transfer during root canal obturation with thermoplasticized gutta-percha. (Pt 1). In vitro heat levels during extrusion. J Endod 1987;13:378-83.

62 Gutmann JL, Rakusin H, Powe R, Bowles WH. Evaluation of heat transfer during root canal obturation with thermoplasticized gutta-percha. (Pt 2).In vivo response to heat levels generated. J Endod 1987:13:441-8.

63 Endal U, Haapasalo M, Zandi H, Coil JM, Eradication of endodontic infection by instrumentation and irrigation solutions. Endod Topic 2005;10:77-102.

64 Shen Y, Haapasalo M, Ricucci D. Reasons for persistent and emerging post-treatment endodontic disease. Endod Topic 2011;18:31-50.

65 Udnes T, Haapasalo M, Endal U. Persistent, recurrent, and acquired infection of the root canal system post-treatment. Endod Topic 2003;6:29-56.

66 Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375-9.

67 Haapasalo M, Ranta H, Ranta KT. Facultative gram-negative enteric rods in persistent periapical infections. Acta Odontol Scand 1983;41:19-22.

68 Hamann C, Rodgers PA, Alenius H, Halsey JF, Sullivan K. Cross-reactivity between gutta-percha and natural rubber latex: assumptions vs.reality. J Am Dent Assoc 2002;133:1357-67.

69 Hammad M, Qualtrough A, Silikas N. Effect of new obturating materials on vertical root fracture resistance of endodontically treated teeth. J Endod 2007;33: 732-6.

70 Hancock 3rd HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001:91:579-86.

71 Hand RE, Smith ML, Harrison JW.Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4:60-4.

72 Harrison JW. Irrigation of the root canal system.Dent Clin North Am 1984;28: 797-808.

73 Hiraishi N, Yau JY, Loushine RJ, et al. Susceptibility of a polycaprolactone-based root canal-filling material to degradation.(Pt 3). Turbidimetric evaluation of enzymatic hydrolysis. J Endod 2007;33:952-6.

74 Holcomb JQ, Pitts DL, Nicholls JI. Further investigation of spreader loads required to cause vertical root fracture during lateral condensation. J Endod 1987;13:277-84.

75 Peters OA, Hulsmann M, Dummer PMH.Mechanical preparation of root canals: shaping goals, techniques and means. Endod Topic 2005;10:30-76.

76 Bakland LK, Ingle JB, Baumgartner J. Ingle's endodontics. Vol. 6. Ontario, Hamilton: BC Decker, Inc., 2008.

77 Taintor JF, Ingle JI, Endodontics. 3rd ed. Philadelphia: Lea & Febiger, 1985.

78 Isci S, Yoldas O, Dumani A. Effects of sodium hypochlorite and chlorhexidine solutions on Resilon synthetic polymer based root canal filling material cones: an atomic force microscopy study. J Endod 2006;32:967-9.

79 Jacobsohn PH, Fedran RJ. Making darkness visible: The discovery of x-ray and its introduction to dentistry. J Am Dent Assoc 1995;126:1359-67.

80 Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod 1994; 20:276-8.

81 Alpert B, Jia WT. Root canal filling material. United States Patent Application 20030113686, June 19, 2003.

82 Jia WT, Jin SH. Self-curing system for endodontic sealant applications. United States Patent Application 20030134933, US Patent & Trademark Office, 2003.

83 Kulild JC, Johnson W. Obturation of the cleaned and shaped root canal system. In: Cohen S, Hargreaves KM, eds. Cohen's pathways of the pulp. St. Louis: Mosby Elsevier, 2011:349-88.

84 Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.

85 Karapinar Kazandag M, Sunay H, Tanalp J, Bayirli G. Fracture resistance of roots using different canal filling systems. Int Endod J 2009;42:705-10.

86 Kuttler Y. A precision and biologic root canal filling technic. J Am Dent Assoc 1958;56:38-50.

87 Lazarski MP, Walker 3rd WA, Flores CM, Schindler WG, Hargreaves KM. Epidemiological evaluation of the outcomes of nonsurgical root canal treatment in a large cohort of insured dental patients. J Endod 2001;27:791-6.

88 Lazdunski A, Guzzo J, Filloux A, Bally M, Murgier M. Secretion of extracellular proteins by Pseudomonas aeruginosa, Biochimie 1990;72:147-56.

89 Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifacio KC, Ito KY, In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics, J Endod 2000;26:391-4.

90 Leonardo MR, Tanomaru Filho M, Silva LA, Nelson Filho P, Bonifacio KC, Ito IY. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod 1999;25:167-71.

91 Lin L, Langeland K. Light and electron microscopic study of teeth with carious pulp exposures. Oral Surg Oral Med Oral Pathol 1981;51:292-316.

92 Loe H, Schiott CR, Karring G, Karring T. Two years oral use of chlorhexidine in man. (Pt 1).General design and clinical effects. J Periodontal Res 1976;11:135-44.

93 Lovato KF, Sedgley CM. Antibacterial activity of endosequence root repair material and proroot MTAagainst clinical isolates of Enterococcus faecalis. J Endod 2011;37:1542-6.

94 Love RM, Enterococcus faecalis--a mechanism for its role in endodontic failure. Int Endod J 2001;34:399-405.

95 Spangberg LSW. Endodontic treatment of teeth without apical periodontitis in essential endodontology prevention and treatment of apical periodontitis.In: Pitt Ford T, Orstavik D. eds. Singapore: Blackwell Munkssgaard Ltd, 2008: 316.

96 Luccy CT, Weller RN, Kulild JC. An evaluation of the apical seal produced by lateral and warm lateral condensation techniques. J Endod 1990;16:170-2.

97 Maeda N, Okamoto M, Kondo K, Incidence of Prevotella intermedia and Prevotella nigrescens in periodontal health and disease. Microbiol Immunol 1998; 42:583-9.

98 Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. Oral Surg Oral Med Oral Pathol 1992;73:729-37.

99 Boopathy T, Mathew S. Enterococcus faecalis-an endodontic challenge. KSR Institute of Dental Science and Research, 33-37.

100 Maus EA, Tan IC, Rasmussen JC, Near-infrared fluorescence imaging of lymphatics in head and neck lymphedema.Head Neck 2012;34:448-53.

101 McMichen FR, Pearson G, Rahbaran S, Gulabivala K. A comparative study of selected physical properties of five root-canal sealers.Int Endod J 2003;36:629-35.

102 Meister F, Jr., Lommel TJ, Gerstein H. Diagnosis and possible causes of vertical root fractures. Oral Surg Oral Med Oral Pathol 1980;49:243-53.

103 Basrani B, Metzger Z, Goodis H. Instruments, materials, and devices.In: Cohen S, Hargreaves KM, eds. Cohen's pathways of the pulp.St. Louis, MO: Mosby Elsevier, 2011:223-82.

104 Metzler KT, Woody RD, Miller AW, 3rd, Miller BH. In vitro investigation of the wear of human enamel by dental porcelain. J Prosthet Dent 1999;81:356-64.

105 Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J 2008;58:329-41.

106 Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1-7.

107 Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475-84.

108 Moorer WR, Genet JM. Antibacterial activity of gutta-percha cones attributed to the zinc oxide component, Oral Surg Oral Med Oral Pathol 1982;53:508-17.

109 Moorer WR, Genet JM. Evidence for antibacterial activity of endodontic gutta-percha cones. Oral Surg Oral Med Oral Pathol 1982;53:503-7.

110 Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod 2004;30:785-7.

111 Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures.Crit Rev Oral Biol Med 2004;15:348-81.

112 Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: (Pt 1). Periapical health. Int Endod J 2011;44:583-609.

113 Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P. Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 2000;64:548-72.

114 Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NAOCl solutions. Int Endod J 2002; 35:934-9.

115 Ohara P, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic irrigants on selected anaerobic bacteria. Endod Dent Traumatol 1993;9:95-100.

116 Oncag O, Hosgor M, Hilmioglu S, Zekioglu O, Eronat C, Burhanoglu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J 2003;36:423-32.

117 Orstavik D. Materials used for root canal obturation: technical, biological and clinical testing. Endod Topic 2005;12:25-38.

118 Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142-9.

119 Ostrander FD. The practice of endodontics: past, present, and future. J Dent Educ 1967;31: 386-8.

120 Pameijer CH, Zmener O. Resin materials for root canal obturation. Dent Clin North Am 2010:54:325-44.

121 Park SY, Shin YP, Kim CH, et al. Immune evasion of Enterococcus faecalis by an extracellular gelatinase that cleaves C3 and Ic3b. J Immunol 2008;181:6328-36.

122 Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NAOCl on vital tissue. J Endod 1985;11:525-8.

123 Pashley EL, Tao L, Pashley DH. Sterilization of human teeth: its effect on permeability and bond strength. Am J Dent 1993;6:189-91.

124 Patterson CJ, McLundie AC, Stirrups DR, Taylor WG. Efficacy of a porcelain refinishing system in restoring surface finish after grinding with fine and extra-fine diamond burs. J Prosthet Dent 1992;68:402-6.

125 Peng L. Ye L, Tan H, Zhou X. Outcome of root canal obturation by warm gutta-percha versus cold lateral condensation: a meta-analysis. J Endod 2007;33;106-9.

126 Peters OA, Schonenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by microcomputed tomography. Int Endod J 2001;34:221-30.

127 Waltimo T, Portenier I, Haapasalo M. Enterococcus faecalis-the root canal survivor and star in post-treatment disease. Endod Topic 2003;6:135-59.

128 Prado M, Gusman H, Gomes BP, Simão RA. The importance of final rinse after disinfection of gutta-percha and resilon cones. Oral Surg Oral Med Oral Pathol, Oral Radiol Endod 2011; 111(6):e21-4.

129 Pupo J, Biral RR, Benatti O, Abe A, Valdrighi L. Antimicrobial effects of endodontic filling cements on microorganisms from root canal. Oral Surg Oral Med Oral Pathol 1983;55:622-7.

130 Radcliffe CE, Potouridou L, Qureshi R, et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J 2004;37:438-46.

131 Ranta K, Haapasalo M, Ranta H. Monoinfection of root canal with Pseudomonas aeruginosa. Endod Dent Traumatol 1988;4:269-72.

132 Rappaport HM, Lilly GE, Kapsimalis P. Toxicity of endodontic filling materials. Oral Surg Oral Med Oral Pathol 1964;18:785-802.

133 Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration.Int Endod J 1995;28:12-8.

134 Regan JD, Fleury AA. Irrigants in non-surgical endodontic treatment. J Ir Dent Assoc 2006;52:84-92.

135 Roane JB, Sabala CL, Duncanson Jr MG. The balanced force concept for instrumentation of curved canals. J Endod 1985;11:203-11.

136 Rocas IN, Siqueira Jr JF, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004;30:315-20.

137 Rolph HJ, Lennon A, Riggio MP, et al. Molecular identification of microorganisms from endodontic infections. J Clin Microbiol 2001;39:3282-9.

138 Rosenthal S, Spangberg L, Safavi K. Chlorhexidine substantivity in root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:488-92.

139 Russell AD, Day MJ. Antibacterial activity of chlorhexidine. J Hosp Infect 1993;25:229-38.

140 Saboia-Dantas CJ, Coutrin de Toledo LF, Sampaio-Filho HR, Siqueira Jr JF. Herpesviruses in asymptomatic apical periodontitis lesions: an immunohistochemical approach, Oral Microbiol Immunol 2007;22:320-5.

141 Sakamoto M, Siqueira Jr JF, Rocas IN, Benno Y. Bacterial reduction and persistence after endodontic treatment procedures. Oral Microbiol Immunol 2007;22:19-23.

142 Sakkal S, Weine FS, Lemian L. Lateral condensation: inside view. Compendium 1991;12:796, 98, 800 passim.

143 Salehrabi R, Rotstein I. Endodontic treatment outcomes in a large patient population in the USA: an epidemiological study. J Endod 2004;30:846-50.

144 Santos SG, Diniz CG, Silva VL et al. Effects of oxidative stress on the virulence profile of Prevotella intermedia during experimental infection in gnotobiotic mice. J Med Microbiol 2007;56:289-97.

145 Sargenti A. Debate on N2: is N2 an acceptable method of treatment? Trans Int Conf Endod 1973;5:176-95.

146 Sathorn C, Parashos P, Messer HH. Effectiveness of single-versus multiple-visit endodontic treatment of teeth with apical periodontitis: a systematic review and meta-analysis. Int Endod J 2005;38:347-55.

147 Schaeffer A, White RR, Walton RE. Determining the optimal obturation length:a meta-analysis of literature. J Endod 2005;31:271-4.

148 Schilder H. Cleaning and shaping the root Canal. Dent Clin North Am 1974;18: 269-96.

149 Schilder H. Filling root canals in three dimensions.Dent Clin North Am 1967;Nov:723-44.

150 Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res 2011;45 Suppl 1: 13-23.

151 Seltzer S, Green DB, Weiner N, DeRenzis F. A scanning electron microscope examination of silver cones removed from endodontically treated teeth. Oral Surg Oral Med Oral Pathol 1972;33:589-605.

152 Serper A, Ucer O, Onur R, Etikan I. Comparative neurotoxic effects of root canal filling materials on rat sciatic nerve. J Endod 1998;24:592-4.

153 Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod 2007;33:96-105.

154 Shipper G, Orstavik D, Teixeira FB, Trope M. An evaluation of microbial leakage in roots filled with a thermoplastic synthetic polymer-based root canal filling material (Resilon).J Endod 2004;30:342-7.

155 Shipper G, Teixeira FB, Arnold RR, Trope M, Periapical inflammation after coronal microbial inoculation of dog roots filled with gutta-percha or Resilon. J Endod 2005;31: 91-6.

156 Siqueira Jr JF. Treatment of endodontic infections. London:Quintessence, 2011.

157 Siqueira Jr JF, Alves FR, Rocas IN. Pyrosequencing analysis of the apical root canal microbiota. J Endod 2011;37:1499-503.

158 Siqueira JR JF, De Uzeda M, Fonseca ME. A scanning electron microscopic evaluation of in vitro dentinal tubules penetration by selected anaerobic bacteria. J Endod 1996;22:308-10.

159 Siqueira Jr JF, Lima KC, Magalhaes FA, Lopes HP, de Uzeda M. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999;25;332-5.

160 Rocas I, Siqueira Jr JF. Microbiology and treatment of endodontic infections in Cohen S, Hargreaves KM, eds. Cohen's pathways of the pulp. St.Louis:Mosby Elsevier, 2011:559-600.

161 Siqueira Jr JF, Rocas IN. Pcr methodology as a valuable tool for identification of endodontic pathogens. J Dent 2003;31:333-9.

162 Siqueira Jr JF, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:85-94.

163 Siqueira Jr JF, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632-41.

164 Sjogren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. J Endod 1990;16:498-504.

165 Sjogren U, Sundqvist G, Nair PN. Tissue reaction to gutta-percha particles of various sizes when implanted subcutaneously in guinea pigs. Eur J Oral Sci 1995; 103:313-21.

166 Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2003;96: 327-31.

167 Smith CS, Setchell DJ, Harty FJ. Factors influencing the success of conventional root canal therapy--a five-year retrospective study. Int Endod J 1993;26:321-33.

168 Smith JB, Loushine RJ, Weller RN. Metrologic evaluation of the surface of white MTA after the use of two endodontic irrigants. J Endod 2007;33:463-7.

169 Soderberg TA, Sunzel BA, Holm S, Elmros T, Hallmans G, Sjoberg S. Antibacterial effect of zinc oxide in vitro.Scand J Plast Reconstr Surg Hand Surg 1990;193-7.

170 Spangberg L. The wonderful world of rotary root canal preparation.Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:479.

171 Spangberg LS, Barbosa SV, Lavigne SD, Ah 26 releases formaldehyde. J Endod 1993;19:596-8.

172 Spradling PM, Senia ES. The relative sealing ability of paste-type filling materials. J Endod 1982;8:543-9.

173 Stavropoulou AF, Koidis PT. A systematic review of single crowns on endodontically treated teeth. J Dent 2007;35:761-7.

174 Stewart GG. The importance of chemomechanical preparation of the root canal. Oral Surg Oral Med Oral Pathol 1955;8:993-7.

175 Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32: 93-8.

176 Stubbs S, Lewis MA, Waddington RJ, Embery G. Hydrolytic and depolymerising enzyme activity of Prevotella intermedia and Prevotella nigrescens. Oral Dis 1996; 2:272-8.

177 Su Y, Wang C, Ye L. Healing rate and post-obturation pain of single-versus multiple-visit endodontic treatment for infected root canals: a systematic review. J Endod 2011;37:125-32.

178 Subramanian K, Mickel AK. Molecular analysis of persistent periradicular lesions and root ends reveals a diverse microbial profile. J Endod 2009;35:950-7.

179 Sundqvist G. Bacteriological studies of necrotic dental pulps (Odontological dissertation No.7). University of Umea, 1976.

180 Sundqvist G, Figdor D. Life as an endodontic pathogen. Ecological differences between the untreated and root-filled root canals. Endod Topic 2003;6:3-28.

181 Takemura N, Noiri Y, Ehara A, Kawahara T, Noguchi T, Ebisu S. Single species biofilm-forming ability of root canal isolates on gutta-percha points. Eur J Oral Sci 2004:112:523-9.

182 Tay FR, Loushine RJ, Weller RJ. Ultrastructural evaluation of the apical seal in roots filled with a polycaprolactone-based root canal filling material. J Endod 2005;31:514-9.

183 Tay FR, Pashley DH. Monoblocks in root canals: a hypothetical or a tangible goal. J Endod 2007;33:391-8.

84 Tay FR, Pashley DH, Loushine RJ, et al. Susceptibility of a polycaprolactone-based root canal filling material to degradation. Evidence of biodegradation from a simulated field test. Am J Dent 2007;20:365-9.

185 Tay FR, Pashley DH, Williams MC, et al. Susceptibility of a polycaprolactone-based root canal filling material to degradation. (Pt 1). Alkaline hydrolysis. J Endod 2005;31:593-8.

186 Tay FR, Pashley DH, Yiu CK, et al. Susceptibility of a polycaprolactone-based root canal filling material to degradation. (Pt 2) Gravimetric evaluation of enzymatic hydrolysis. J Endod 2005;31:737-41.

187 Teixeira FB, Teixeira EC, Thompson J, Leinfelder KF, Trope M. Dentinal bonding reaches the root canal system. J Esthet Restor Dent 2004;16:348-54; discussion 54.

188 Teixeira FB, Teixeira EC, Thompson JY, Trope M. Fracture resistance of roots endodontically treated with a new resin filling material. J Am Dent Assoc 2004; 135: 646-52.

189 Thomas RZ, Ruben JL, ten Bosch JJ, Huysmans MC. Effect of ethylene oxide sterilization on enamel and dentin demineralization in vitro. J Dent 2007;35:547-51.

190 Tommassen J, Filloux A, Bally M, Murgier M, Lazdunski A. Protein secretion in Pseudomonas aeruginosa, FEMS. Microbiol Rev 1992;9:73-90.

191 Topuz O, Saglam BC, Sen F, Sen S, Gokagac G, Gorgul G. Effects of sodium hypochlorite on gutta-percha and Resilon cones: an atomic force microscopy and scanning electron microscopy study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112: e21-6.

192 Walton RE, Torabinejad M. Endodontics principles and practice. 4th ed. St. Louis: Mosby Elsevier, 2002.

193 Walton RE, Torabinejad M. Endodontics: principles and practice.4th ed. St. Louis: Mosby Elsevier, 2009.

194 Toutain-Kidd CM, Kadivar SC, Bramante CT, Bobin SA, Zegans ME. 'Polysorbate 80 inhibition of Pseudomonas aeruginosa biofilm formation and its cleavage by the secreted lipase lipa. Antimicrob Agents Chemother 2009;53:136-45.

195 Tsao TF. Endodontic treatment in China. Int Endod J 1984;17:163-75.

196 Venturi M, Breschi L. Evaluation of apical filling after warm vertical gutta-percha compaction using different procedures. J Endod 2004;30:436-40.

197 Vianna ME, Conrads G, Gomes BP, Horz HP. Identification and quantification of archaea involved in primary endodontic infections. J Clin Microbiol 2006;44: 1274-82.

198 Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. In Vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:79-84.

199 von Woedtke T, Julich WD, Thal S, Diederich M, Stieber M, Kindel E. Antimicrobial efficacy and potential application of a newly developed plasma-based ultraviolet irradiation facility. J Hosp Infect 2003;55:204-11.

200 Wagner VE, Gillis RJ, Iglewski BH, Transcriptome analysis of quorum-sensing regulation and virulence factor expression in Pseudomonas aeruginosa.Vaccine 2004; 22:(Suppl 1) S15-20.

201 Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of nitinol root canal files. J Endod 1988;14:346-51.

202 Warneke S, Arenskotter M, Tenberge KB, Steinbuchel A. Bacterial degradation of poly(trans-1,4-isoprene) (gutta-percha). Microbiol 2007;153:347-56.

203 White HE, Goodis S,. Marshall J, Marshall GW. Sterilization of teeth by gamma radiation. J Dent Res 1994;73:1560-7.

204 White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod 1997;23:229-31.

205 Wu MK, Wesselink PR. Endodontic leakage studies reconsidered. (Pt 1). Methodology, application and relevance. Int Endod J 1993;26:37-43.

206 Zamany A, Safavi K, Spangberg LS. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:578-81.

207 Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.

208 Zhu X, Wang Q, Zhang C, Cheung GS, Shen Y. Prevalence, phenotype, and genotype of Enterococcus faecalis isolated from saliva and root canals in patients with persistent apical periodontitis.J Endod 2010;36:1950-5.

ABSTRACT

BIODEGRADABILITY OF RESILON, A RESIN-BASED ROOT CANAL OBTURATING MATERIAL, BY TYPICAL ENDODONTIC PATHOGENS

by

Ashleigh M. Rexford

Indiana University School of Dentistry
Indianapolis, Indiana

Root canal therapy is a recommended treatment for apical periodontitis. Root canal failure can occur as a result of microbial leakage. In an attempt to minimize leakage, the product Resilon, a resin-based root canal obturating cone material, was introduced in 2004. The resin sealer bonds to both the core material and to the dentin of the canal walls. Resilon has no bactericidal or antimicrobial effect.[15] Furthermore, it has been shown that Resilon is susceptible to alkaline and enzymatic hydrolysis as well as bacterial degradation.[73,184-186] It has been suggested that Resilon may be susceptible to degradation by microorganisms found in the infected root canal space. This work focuses on the susceptibility of root canal obturating materials to degradation by endodontic pathogens seen in root canal-treated teeth with apical periodontitis. The aim of this study was to determine if Resilon could be degraded by selected pathogenic bacteria found in

the infected root canal system, and if this degradation is more severe than with gutta-percha, a conventional obturating material.

P. intermedia, *E. faecalis* and *P. aeruginosa*, known endodontic pathogens, were inoculated on discs of obturating material (Resilon or gutta-percha) mounted on a platform and placed in wells containing TSB incubated at 37°C under aerobic conditions. The discs were polished, examined by SEM, profilometry, and elemental analysis prior to inoculation to establish a baseline and were then re-examined by these methods one month after inoculation. The overall results were inconclusive; and using these methods, it cannot be determined that the selected bacteria can degrade Resilon. An ideal future study would utilize SEM with gold-coated samples as well as atomic force microscopy to evaluate for changes in topographical features of these obturating materials. A notable finding was that Resilon turns black when exposed to bacteria, and the significance of this finding should be addressed in future studies.

CURRICULUM VITAE

Ashleigh Marie Rexford

August 2001 to May 2005	BS, Biology James Madison University Harrisonburg, VA
August 2005 to May 2009	DMD Tufts University School of Dental Medicine Boston, MA
July 2009 to July 2010	General Practice Residency Indiana University Wishard Hospital Indianapolis, IN
July 2010 to Present	MSD, Endodontics Indiana University School of Dentistry Indianapolis, IN

Professional Organizations

American Dental Association (ADA)
American Association of Endodontists (AAE)
Indiana Dental Association (IDA)