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Research Article

In Vitro Evaluation of the Effect of a Warm or Cold Obturation Technique on Apical Microleakage with Various Bioceramic Sealers

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Abstract

Background: Research has shown that heat from warm obturation techniques can affect properties of bioceramic sealers, such as altering setting time, flow, and film thickness. However, no study has shown if these heat-induced changes can influence their apical sealing ability. Therefore, this study aims to determine if obturation technique, particularly a warm technique, can influence the apical sealing ability of various bioceramic sealers. The hypothesis is that the obturation technique will not influence the apical sealing ability of the tested sealers.

Methods: Ninety-six extracted single-rooted human teeth were decoronated, cleaned, shaped, and randomly divided into eight groups (n = 12) based on obturation technique and sealer. There was one positive control, one negative control, and six experimental groups. Obturation included either single cone or warm vertical condensation technique with Brassler's BC Sealer, BC Sealer HiFlow, or recently released BC Sealer Ion+. After obturation, roots were sealed with nail polish (except the apical foramen), submerged in methylene blue dye, and cleared using a decalcification and dehydration protocol. Dye penetration was measured from the apical foramen to the deepest coronal extent using ImageJ software. Statistical analysis was performed using multiple student's t-tests for all group combinations ($p < 0.05$).

Results: Results demonstrated that the positive control group showed significantly more apical dye leakage when compared to all other groups, with average dye leakage of 177.81 ± 82.75μm. No significant differences in apical dye penetration were observed amongst the six experimental groups.

Conclusions: Within the limitations of this study, it is considered safe to utilize a warm obturation technique with bioceramic sealers marketed for both warm and/or cold obturation techniques. These findings demonstrate that the studied bioceramic sealers can be safely used with either a cold or warm obturation technique, expecting the apical seal to be adequate with any combination of these sealers or obturation techniques utilized in this study.

Keywords: *Dye Leakage, Bioceramic Sealer, Single Cone, Warm Vertical Condensation*

Introduction

The need for an endodontic sealer in addition to gutta percha was proven in Marshall and Massler's study in 1961 with radioisotopes, which showed how gutta percha and sealer provided a complete seal to the marginal ingress of small radioisotopes [1]. Since then, gutta percha has been commonly used as the core obturating material in addition to an endodontic sealer to provide a three dimensional obturation of the root canal space following chemo-mechanical canal preparation utilizing two common obturation techniques: the cold technique of single cone obturation and the warm technique of warm vertical condensation.

Single cone obturation involves the placement of a master gutta percha cone to working length with sealer, followed by removal of excess coronal gutta percha to the cemento-enamel junction with a heated endodontic instrument. This technique relies on endodontic sealer to fill the majority of canal irregularities, especially those canals with ribbons and fins [2]. Single cone obturation can reach approximately 90% success [3,4], and studies have shown no difference in long-term success between warm vertical and single cone techniques [5].

The warm vertical condensation technique developed by Schilder, was created to help fulfill his main objective of root canal obturation in three dimensions [6]. This idea fulfills one of the core principles of the endodontic triad in thorough debridement, disinfection, and complete obturation of the root canal system [7,8]. This technique involves placement of a master gutta-percha cone with sealer to working length. Gutta-percha is then heated and removed with a heated plugger to approximately 4-5mm short of the working length. Hand pluggers are used to vertically compact the gutta-percha/sealer apically and into canal irregularities, while the reminder of the canal is filled with heated gutta-percha and vertically compacted in 3-4mm increments [6]. Modifications to this technique such as the continuous wave technique popularized by Buchanan, have been developed over the years but rely on the same core principles [9].

Various formulations of endodontic sealers have been developed, but still, no sealer meets all of the ideal properties set forth by Grossman. Zinc oxide and eugenol, calcium hydroxide, glass ionomer, silicone, epoxy resin, and bioceramic materials are some of the most common formulations used in endodontics [10]. However, no sealer has been developed that meets all of the eleven ideal properties set forth by Grossman which has led to the continued development of sealers over the years [11].

Recently, calcium silicate based sealers (bioceramic sealers) have become some of the most commonly used sealers in endodontics today due to favorable characteristics such as exceptional biocompatibility, high flow, fast setting time, high pH, and antimicrobial potential [10,12]. In particular, Giacomino et al. 2019, stated that cell death with Roth's sealer was seen at concentrations 100x higher than with Endosequence BC sealer, which additionally exhibited enhancement to osteoblastic differentiation [13]. Therefore, research investigating "new to market" sealers is of utmost importance due to the variety of available calcium silicate sealers on the market today.

Traditional Endosequence BC Sealer is marketed for use with a cold obturation technique only, while the lower viscosity version, EndoSequence BC HiFlow, is marketed for use with warm obturation techniques. Alternatively, recently released BC Sealer Ion+ is marketed as a sealer which can be used with either a cold or warm obturation technique.

During a literature review, previous research has shown that heat from warm obturation techniques can affect physical properties of bioceramic sealers, such as accelerating setting time, reducing flow, and increasing film thickness [14]. However, no previous studies were discovered which have investigated the potential impact that heat-induced changes may have on the apical seal obtained with these calcium silicate sealer variations. Investigating any potential impacts to the apical seal obtained with these sealers based on the use of heat could provide insight for optimal sealer selection based on the planned obturation technique.

Therefore, this study aims to determine if the obturation technique, particularly a warm technique, can influence the apical sealing ability of Endosequence BC Sealer, BC Sealer HiFlow, and BC Sealer Ion+ through a leakage test utilizing methylene blue dye, microscopic examination, and a demineralization, dehydration, and clearing process.

Methods

Sample Selection: Ninety-six extracted single-rooted human teeth were selected for this study. Teeth were de-identified and collected from various dental clinics at the LSU School of Dentistry and private dental practice offices. Teeth were stored in jars containing 10% formalin. Inclusion criteria included teeth with a single root canal system. Caries or restorations were only permitted in the crown of the teeth studied. Teeth with visible root fractures, root caries, or root resorption extending apical to a measurement 15mm from the apex were excluded. All teeth were measured from the apex to a length of approximately 15mm and were decoronated to this level leaving 15mm root segments.

Cleaning and Shaping: Prior to instrumentation, all teeth were soaked in saline for 24 hours. Each pulp chamber/canal was accessed with a #557 dental bur utilizing a high speed dental handpiece if the pulp chamber/canal had not yet been exposed through decoronation. A #10 K-file was placed into each canal until the tip of the file was visible at the apical foramen under a 0.8x magnification setting using a Zumax 2380 dental operating microscope. This length was measured, and 1mm was subtracted to provide a working length measurement. Each tooth was cleaned and shaped with the ProTaper Ultimate rotary sequence to a master file size of the Finisher F3 (30.009v) using a Kerr Elements Connect Cordless Motor set to the manufacturer's recommended settings of 400rpm and 4 Ncm. Throughout the cleaning and shaping process, canals were irrigated between each file with 1mL of 6% sodium hypochlorite (NaOCl) using a 30G side-vented irrigation needle. The final irrigation protocol involved the use of 2mL NaOCl, then 2mL 17% ethylenediaminetetraacetic acid (EDTA), followed by 2mL of NaOCl with 30 seconds of sonic activation completed between each irrigation step utilizing the EndoActivator.

Groups: Teeth were randomly divided into eight groups (n = 12) based on the obturation technique and sealer utilized. Included was a positive control, negative control, and six experimental groups.

Group 1: Nail polish only (negative control) (NP)

Group 2: Gutta percha only without sealer obturation (positive control) (GPNS)

Group 3: Gutta percha and BC Sealer with single cone obturation (BC-SC)

Group 4: Gutta percha and BC Sealer with warm vertical condensation obturation (BC-WVC)

Group 5: Gutta percha and BC HiFlow Sealer with single cone obturation (BCH-SC)

Group 6: Gutta percha and BC HiFlow Sealer with warm vertical condensation obturation (BCH-WVC)

Group 7: Gutta percha and BC Sealer Ion+ with single cone obturation (BCI-SC)

Group 8: Gutta percha and BC Sealer Ion+ with warm vertical condensation obturation (BCI-WVC)

Obturation: Following final irrigation, canals were dried using DiaDent size medium sterile, absorbent paper points until appearing dry. For the negative control group, no obturation was completed. For the positive control group, only a master gutta percha cone was used for obturation. In the treatment groups, obturation was completed with conform fit gutta percha cones matching the ProTaper Ultimate Finisher F3 file along with BC Sealer, BC Sealer HiFlow, or BC Sealer Ion+. Gutta percha cones were measured, placed to working length, and adjusted as necessary to ensure a fit to the working length. Sealer was injected into the coronal half of the prepared root canal space, and a gutta percha cone was gently placed to working length, removed a few millimeters, and finally seated to working length. The Gutta-Smart Cordless Obturation system was utilized to sear off excess gutta percha in the single cone group, and cold hand pluggers were used to condense the coronal segment to a level approximately 2-3mm short of the orifice. In the warm vertical condensation group, the master gutta percha cone was seared to a level approximately 4mm short of working length and vertically condensed. The Gutta-Smart backfill unit was then used with a 25G gutta-percha cartridge to introduce heated gutta percha at 160°C, which was also vertically condensed to a level approximately 2-3mm apical to the orifice. The coronal segment was then sealed with white BC liner and light-cured following manufacturer recommendations. Teeth were then stored for at least 7 days at 37°C in a constant humidity chamber.

Leakage Test: For Group 1 (NP), the entire surface of the tooth was coated in 2 coats of nail polish (Sally Hansen Insta Dri Nail Color Nail Polish - Green). For all other groups, 2 coats of nail polish covered all but the apical 2mm of the root to ensure the apical foramen remained exposed for apical leakage assessment. Teeth were then submerged in a solution of 1% methylene blue dye for 72 hours. After rinsing off excess dye with saline, nail polish was removed with cotton rounds and non-acetone nail polish remover.

Demineralization/Dehydration/Clearing: The following clearing technique process was described by Robertson et al. but lengthened slightly to ensure complete demineralization, dehydration, and clearing [15]. Each tooth was demineralized by soaking in 5% nitric acid for 10 days, with daily changes of the acid, followed by 4 hours of submersion in running tap water. Demineralized teeth were then placed in PBS for 24 hours. The teeth were then dehydrated in ascending percentages of ethyl alcohol. The samples were placed in 70% ethyl alcohol for 24 hours, followed by 95% ethyl alcohol for 24 hours, and finally in 100% ethyl alcohol for 24 hours. To complete the clearing process, the roots were submerged in methyl salicylate for 24 hours.

Leakage Assessment: Using the Zumax dental operating microscope at a magnification setting of 0.8x, an image of each cleared tooth was taken for assessment of apical leakage at a set image acquisition distance. The images were uploaded into ImageJ software for analysis. Linear measurements of apical dye penetration in pixels extending from the apical foramen to the deepest coronal extent of leakage were recorded. Two examiners were used to determine leakage measurements. Examiner agreement was necessary to record measurements, and with any discrepancy, a third examiner was consulted to assist in measurement determination. A reference image of a periodontal probe was used to convert pixel measurements to millimeter measurements following data collection. The mean and standard deviation for each group's measurements were determined.

Statistical Analysis: Statistical analysis was performed using a student's t-tests ($p < 0.05$) to determine significant differences between all groups using Microsoft Excel. Individual t-tests were conducted for all group combinations.

Results

Apical dye leakage was found to be the greatest in the positive control, Group 2 (GPNS), in which obturation was completed with only gutta percha. In this group, 10/11 (90.91%) teeth depicted apical dye leakage. Only eleven samples were included in this group for analysis as one sample was excluded due to fracture of the root tip during the experimental period. Average dye leakage in this group was 177.81 ± 82.75μm.

Of the remaining groups including the negative control (NP) and all experimental groups, an average of 2/12 samples per group depicted apical dye leakage. Average dye leakage of these groups ranged from 13.36-46.64 ± 36.56-147.54μm. There was no significant difference noted between any of these groups ($p>0.05$).

A significant difference was noted between Group 2 and all other groups. P-values were <0.05 for comparisons of Group 2 to Groups 1, 3, and 8, and P-values were <0.001 for comparisons of Group 2 to Groups 4, 5, 6, and 7.

Figure 1 displays representative images of sample teeth with and without apical dye leakage, while Figure 2 depicts a graph of the average apical dye leakage per study group with standard deviation.

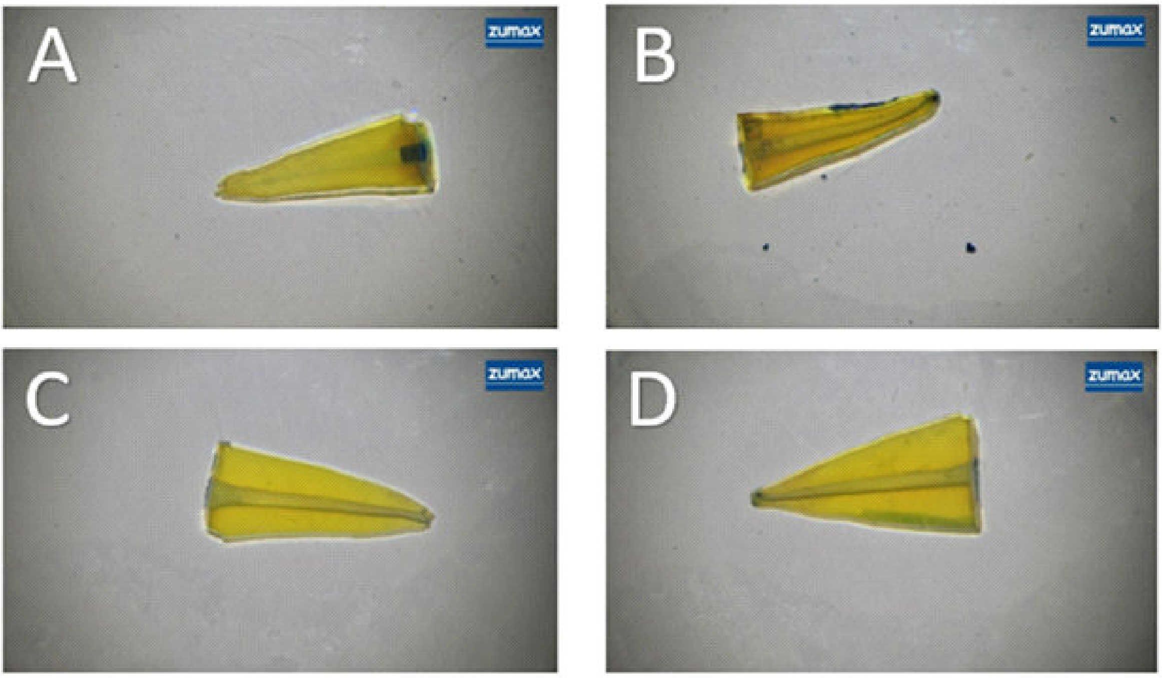

Figure 1. Images representing samples with and without dye leakage. Image A depicts the negative control group, Group 1 (NP), representing no apical dye leakage. Coronally, nail polish remains at the orifice. Image B represents the sample with the greatest recorded leakage from Group 2 (GPNS), the positive control, obturated with gutta percha only. Image C depicts an experimental tooth obturated from Group 3 (BC-SC) depicting no apical leakage and obturation material into apical canal anatomy. Image D represents a sample from Group 4 (BC-WVC) depicting the typical extent of apical dye leakage, amongst the experimental groups.

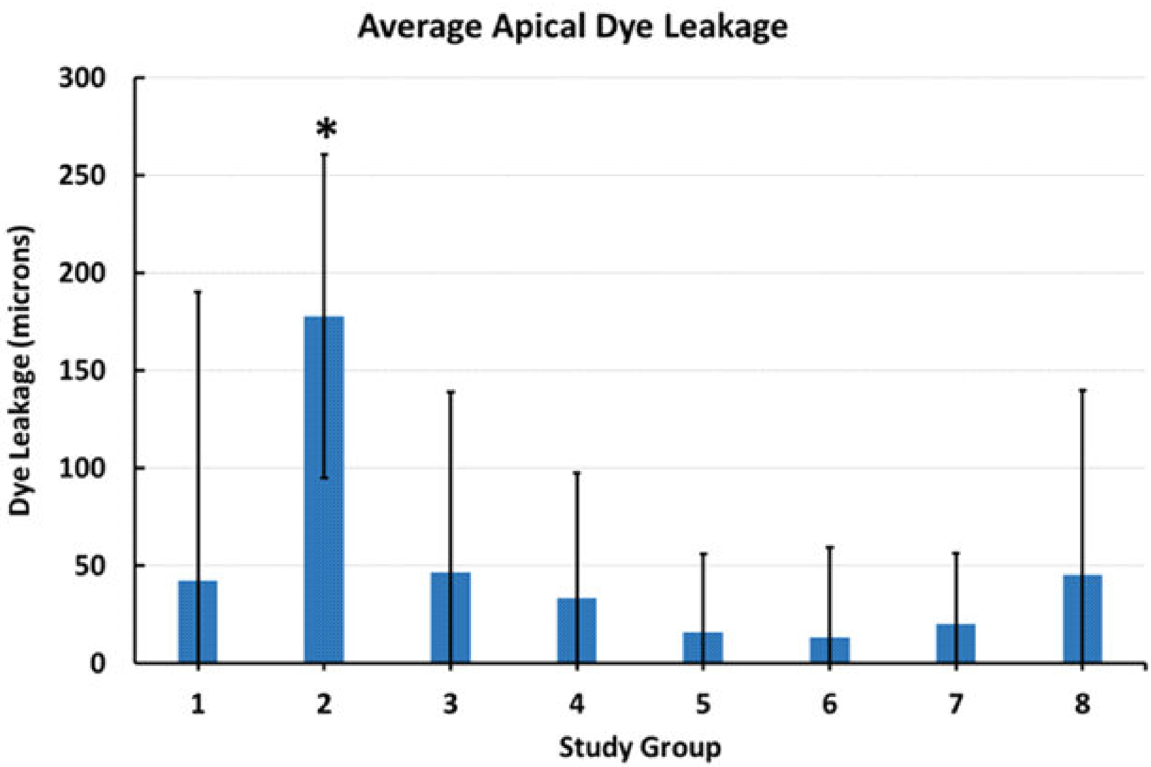

Figure 2. Average apical dye leakage per study group (microns) with standard deviation. The asterisk signifies the only significant difference found, which was between Group 2 and all other study groups.

Discussion

As we have known for decades, endodontic sealers are utilized to help provide a complete, three-dimensional obturation of the root canal system [6]. The importance of this being popularized in the 1960s through works by Ingle, Seltzer and Bender, Schilder, and others [6,7,8]. In particular with endodontic sealers, Marshall and Massler's 1961 study, demonstrated how endodontic sealers are immensely important in providing the complete apical seal of the root canal system by blocking ingress of radioisotopes [1].

With the continued development of endodontic sealers over time, it is important to continue to evaluate these new products to ensure materials and techniques are maintaining or improving upon historical results and success rates.

In the past couple decades, many studies have documented the improved biocompatibility of bioceramic sealers over more conventional sealers in the form of epoxy-resin or zinc oxide and eugenol sealers. These bioceramic sealers have shown similar physical characteristics, higher biocompatibility with less cytotoxicity, enhancement of osteoblastic differentiation and less impact on inflammatory function of macrophages. [12,13]

Early bioceramic sealers were initially intended for use with cold obturation techniques, relying on moisture in the dentinal tubules to begin the setting reaction. However, the application of heat may impact the moisture content within the root canal system and lead to changes in the handling properties of these sealers such as reducing flow and increasing film thickness, and can impact the setting reaction by accelerating setting time [10]. A review by Ashkar also discussed how studies have shown that heat can accelerate setting time, reduce flow, and increase film thickness of certain bioceramic sealers. Therefore, high-flow bioceramic sealers were created and marketed to be used with warm obturation techniques as seen in Ashkar's study where Endosequence BC Sealer Hi-flow showed the ability to resist some of the effects that heat can induce on bioceramic sealers [14].

Due to these observations, this study aimed to investigate if obturation technique, particularly a warm technique, would influence the apical sealing ability of Endosequence BC Sealer, BC Sealer HiFlow, and BC Sealer Ion+ through an apical dye leakage test with methylene blue dye. Detection of dye leakage after clearing the teeth provides evidence of a breakdown of the seal in the apical third of the root canal. The goal of this study was to help clinicians select the most appropriate bioceramic sealer based on their planned cold or warm obturation technique by determining if heat has an impact on the apical seal.

The results of this study further support the importance of utilizing an endodontic sealer in conjunction with gutta percha to provide an adequate apical seal during root canal treatment. This was proven by a significantly greater length of apical dye leakage noted in the positive control group (GPNS), when compared to all other groups. This group showed an average apical dye leakage of 177.81 ± 82.75μm, while the remaining treatment groups and negative control group (NP) showed a range of apical dye leakage from 13.36-46.64 ± 36.56-147.54μm. There was no significant difference in dye leakage noted between Groups 1,3,4,5,6,7, and 8. However, a significant difference was noted between Group 2 (GPNS) and all other groups.

These results also provide two important findings. First, it was shown that the application of heat did not appear to affect the apical sealing ability of the various formulations of the bioceramic sealers used in this study. Sealers intended for a cold obturation technique (BC Sealer and BC Sealer Ion+) showed no significant difference in apical dye leakage when compared to BC Sealer Hi-flow when they were used for warm vertical condensation technique. Conversely, when used in a cold obturation technique, BC Sealer Hi-flow also showed no significant difference in apical dye leakage when compared to BC Sealer and BC Sealer Ion+. A secondary finding demonstrated that, without sealer, the apical seal appears to be incomplete. This was an expected and known outcome which was proven again through this dye leakage study.

These findings demonstrate that the studied bioceramic sealers can be safely used with either a cold or warm obturation technique, expecting the apical seal to be adequate with any combination of the sealers or obturation techniques utilized in this study. Clinicians do not need to be concerned with an alteration of the apical seal when using these bioceramic sealers with warm obturation techniques. However, it is documented and expected that heat will induce changes in physical properties which may impact handling of these materials.

The main limitation to this study is that apical dye leakage tests have a limited correlation with clinical performance. The presence of apical leakage is not a predictor of an unsuccessful clinical outcome, as various factors in this leakage model exist that may limit its reliability. In this study, methylene blue dye was used due to its particle size being smaller than that of bacteria. Leakage with this dye can indicate the leakage potential of nutrients and other substances into the root canal system which can be used by residual bacteria to sustain bacterial activity. However, this study does provide initial insight into the apparent lack of an effect that heat has on the apical sealing ability of bioceramic sealers. Further studies including fluid filtration or glucose penetration models with advanced imaging such as confocal laser scanning microscopy or micro-computed tomography can be considered for evaluation of apical dye penetration.

Conclusions

This *in vitro* study assessed how the apical sealing ability of various bioceramic sealers may be affected by the use of a warm or cold obturation technique through an apical dye microleakage model. Results demonstrated that equivalent and adequate apical seals are expected for all of the studied bioceramic sealers with either a warm or cold obturation technique. Heat may alter the physical and handling properties of these sealers, but the apical sealing ability appears to be unchanged. Therefore, it appears that conventional and high-flow formulations of bioceramic sealers can be safely used with either a cold or warm obturation technique.

Conflict of Interest

The authors declare no conflict of interest.

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