 DOI: 10.21608/adjalexu.2024.266923.1475

APICAL MICROLEAKAGE OF ROOT END RESECTED TEETH AFTER ORTHOGRADE OBTURATION USING A BIOCERAMIC SEALER: AN IN VITRO BACTERIOLOGICAL STUDY

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ABSTRACT

BACKGROUND: Endodontic surgery becomes a practical choice when non-surgical interventions are ineffective or are not expected to yield improved results. Limited access and technical challenges may prevent the implementation of controlled retrograde procedures in certain teeth.

AIM OF THE STUDY: The objective was to compare bacterial microleakage of root end resected teeth after orthograde obturation using a single cone technique with a bioceramic sealer versus MTA retrofilling.

MATERIALS AND METHODS: Thirty human extracted maxillary incisors were prepared and divided randomly into three groups. In group1, the canals were filled using the single cone technique with bioceramic sealer, followed by root resection 3 mm from the apex. Group 2 received standard retrograde ultrasonic preparation and retrograde obturation with mineral trioxide aggregate (MTA) and group 3 negative control. Subsequently, the roots were placed in a sterile experimental model and filled coronally with E. faecalis bacterial suspension for 30 days. Turbidity and the time taken for turbidity in the broth were analyzed to assess bacterial microleakage from the canal.

RESULTS: The study revealed that there was no statistically significant difference in bacterial microleakage observed between the two test groups.

CONCLUSION: Performing root end resection following a single cone technique obturation, along with a bioceramic sealer, may offer a favorable alternative approach when compared to the established gold standard MTA retrograde obturation.

KEYWORDS: Single cone technique, bioceramic sealer, apical surgery, bacterial microleakage, MTA.

RUNNING TITLE: Microleakage of single cone technique and root resection.

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INTRODUCTION

If nonsurgical root canal treatment and retreatment prove ineffective, endodontic surgery emerges as a viable therapeutic alternative [1]. Periapical surgery is designed to eliminate infected tissues and establish a tight apical seal, preventing the escape of residual irritants into the periradicular area [2].The effectiveness of endodontic surgery is believed to hinge significantly on the achievement of a secure apical seal [3].

The use of root-end filling material is crucial in endodontic surgical procedures. Ideally, the material should be biocompatible and non-irritating to the host and surrounding tissues. It should also be non-resorbable, possess antibacterial properties released gradually over time, be radiopaque, dimensionally stable, non-corrosive, resistant to humid environments, and have regenerative capabilities for the periodontal ligament. In addition to these characteristics,

retrograde filling is expected to effectively encapsulate any remaining bacteria in the dentinal tubules and root canal, ultimately leading to bacterial death [4,5].Various substances have been employed in contemporary endodontic surgery for root-end filling, including intermediate restorative material (IRM), mineral trioxide aggregate (MTA), Biodentine, and more recently developed bioceramic root repair materials.

Since being introduced in the 1990s, mineral trioxide aggregate (MTA) has become widely recognized as the preferred retrograde filling

material for apical surgeries. This is attributed to its exceptional sealing ability, biocompatibility, favorable physical and chemical properties, and its capability to stimulate the formation of hard tissue [6]. Nonetheless, MTA possesses certain drawbacks, such as challenging handling properties, a high economic burden, limited resistance to compression and flexion, a significant metal content that may lead to discoloration of the treated tooth. Furthermore, its extended setting time may lead to material washout in a damp surgical environment [6,7].

NeoMTA® 2 (NEO; Avalon Biomed Inc.) represents a novel formulation of calcium silicate-based cement, exhibiting comparable chemistry to MTA Plus, ProRoot MTA, and MTA Angelus. Notably, it is finely ground, making it easier to handle, with a smoother consistency and enhanced color stability, thanks to the addition of tantalum oxide (Ta2O5) as a radiopacifier [8].

Over the past few years, the single cone technique (SCT) utilizing bioceramic sealers has become increasingly popular in endodontics, mainly due to their outstanding biological and physiochemical features [9]. In this approach, the root canal is filled with a single cone that corresponds to the size of the final rotary file employed for shaping the root canal and a bioceramic sealer. Consequently, the single cone technique is considered a straightforward and less dependent on operator skill, offering effective sealing properties attributed to the exceptional flowability of bioceramic sealer [10,11].

NeoSEALER Flo, produced by Avalon Biomed in Houston, TX, USA, is a newly formulated calcium silicate sealer that comes in an injectable and pre-mixed form. According to Fausto Zamparini and colleagues, NeoSealer Flo has been identified as a recommended bioceramic sealer due to its adherence to essential chemical and physical standards. Additionally, it releases biologically relevant ions, leading to the development of a discernible calcium phosphate (CaP) layer [12].

Various methodologies can assess apical microleakage, including dye leakage, fluid filtration, and bacterial leakage. Despite the dye penetration method being widely preferred and frequently utilized, it has significant limitations. These limitations include conflicting and contradictory findings, influenced by factors like dye type and pH, as well as tooth specimen storage medium, such as formalin [13]. Another notable drawback is the small size of dye molecules compared to bacteria [14]. Additionally, dye penetration lacks uniformity around the margins of the root-end filling [15]. Consequently, some researchers argue that dye penetration methods are unreliable, and the clinical relevance of these methods remains questionable. Using this underlying concept, the current research employed the bacterial microleakage test.

If effective sealing is achieved through root-end resection following orthograde obturation using the single cone technique and a bioceramic sealer, without the need for retrograde obturation, it can address certain difficulties encountered in the process of retrograde preparation and filling such as difficult accessibility, anatomical intricacies, procedure duration, and clinician expertise [16].

Hence, the study aimed to compare bacterial microleakage of orthograde obturation using the single cone technique and a bioceramic sealer, followed by root end resection without performing retrograde obturation versus MTA retrograde obturation.

The null hypothesis posited that there would be no noteworthy distinctions in bacterial microleakage among the evaluated techniques.

MATERIALS AND METHODS

1 .Sample Preparation

The research plan obtained approval from the Research Ethics Committee at the Faculty of Dentistry, Alexandria University (IRB No. 001056 – IORG 0008839). To maintain an 80% power level, the initial sample size of 9 per group was adjusted to 10, considering potential errors during laboratory processing, as determined by a power analysis using G*Power Version 3.1.9.4 [17,18].

This study involved thirty human extracted maxillary incisors within the last six months due to severe periodontal disease with a single straight canal (curvature of <5 degrees) [19]. Teeth with root caries or resorption, stored previously in formalin solution, with no apical patency,with an apical diameter of more than k-file #25 and teeth with dilacerated and severely curved roots were excluded from the study.

Digital X-rays, captured from different angles, confirmed the presence of a sole canal. The teeth were cleaned of remaining deposits and periodontal tissues using curette, then preserved in a solution containing 0.1% thymol.

The crowns of the included teeth were cut to achieve a 16-mm sample length. The working length was determined using a #15 stainless steel K-file (Mani, Nakanishi Inc., Tokyo, Japan), inserted into the root canal until barely visible at the apical foramen. Subtracting 1mm from this point established the measurement [20].

After establishing the mechanical glide path, canal shaping used HyFlex rotary files (Coltene/Whaledent AG, Altstatten, Switzerland) up to size 40 with a .04 taper (500 rpm; 2.5 Ncm). Adequate irrigation occurred throughout shaping procedure using a 2.5% sodium hypochlorite solution [21]. Following biomechnical preparation, the canals were flushed with 3ml of 17% EDTA [21], then 3ml of 2.5% sodium hypochlorite to eliminate the smear layer. A final rinse with 2 mL

of sterile saline was ensured complete removal of NaOCl and EDTA [22]. Canals were dried using three sterile paper points, each with a size of 40/0.04(Hyflex EDM paper points, Coltene/ Whaledent).

The samples were autoclaved for sterilization before random allocation into three groups. For groups 1 and 2, each canal was filled using the single cone technique, employing a matched gutta-percha cone size 40/0.04 (Hyflex EDM gutta-percha points, Coltene/Whaledent) and NeoSEALER Flo (Avalon Biomed, Houston, TX, USA). Subsequently, the cone was trimmed at the orifice level with a heated instrument. After a one-week storage period in an incubator at 100% relative humidity and 37°C, the apical 3 mm were resected using a surgical bur (SS White Zekrya Bur) [23].

In group 1, roots were solely resected without additional retrograde preparation and filling. In contrast, for group 2, a root-end preparation was carried out 3 mm into the root canal space using an ultrasonic retro-tip (NSK VarioSurg3 Retrograde Endo Tip E32D-S). Subsequently, MTA cement (NeoMTA® 2 Avalon Biomed, Houston, TX, USA) was condensed to the cavities with a microplugger. Following these procedures, radiographs were taken to verify uniform filling. Subsequently, samples were placed in a humidified chamber at a relative humidity of 100% and a temperature of 37ºC for 7 days to ensure complete setting of the MTA.

For groups 1and 2, the external surfaces of the samples were sealed with two layers of nail polish, excluding the prepared apical areas to prevent bacterial leakage through accessory canals.

Group 3 (negative control): Samples were intentionally left without retrograde preparation and filling. The entire root surface, including the apical portion, was covered with two layers of nail polish and utility wax [3].

The Experimental Apparatus:

The experimental setup involved mounting all samples in accordance with a previously described experimental model [24] (Figure 1). Specifically, the samples were inserted into 1.5 mL microcentrifuge tubes. To ensure a secure fit, the gaps between the tube and the root were sealed using cyanoacrylate adhesive and nail varnish. Subsequently, the assembled chamber underwent overnight sterilization with ethylene oxide gas. Following sterilization, the microcentrifuge tube was placed inside a sterile glass tube filled with sterile brain heart infusion (BHI) broth (HiMedia Laboratories Pvt. Ltd, India). The junctions between the microcentrifuge tube and the rubber cap of the glass vial were sealed using cyanoacrylate adhesive [21]. The designed apparatus was then incubated at 37°C for 24 hours to verify sterility, ensuring that approximately 2 mm of the root apex was immersed in the broth [21].

Bacterial Contamination of the Apparatus:

Testing utilized Enterococcus faecalis (E. faecalis) (ATCC 29212), with the bacteria being maintained through subculturing on BHI agar. Prior to testing, colonies were aseptically transferred to BHI broth and incubated at 37°C for 24 hours. The E. faecalis suspension in BHI broth was adjusted to a density corresponding to a 0.5 McFarland standard using a McFarland densitometer [17].

Subsequently, a 40 µL aliquot was applied to the coronal part of the roots. The apparatus was then incubated at 37°C with 100% humidity. Following sterile technique, 40 µL of fresh media was added to the coronal part of each sample in the two test groups group every 3 days to maintain bacterial viability. The negative control group was inoculated with BHI broth alone, devoid of bacteria.

Sample Evaluation:

Bacterial microleakage was monitored by assessing the cloudiness of the BHI broth daily for 30 days following the introduction of E. faecalis into root canals. The time taken for leakage (in days) was determined and documented. To confirm the presence of E. faecalis, a 100 µL sample from the cloudy broth in the glass tube was plated onto blood agar plates and incubated aerobically at 37°C overnight through quantitative culture. The confirmation of E. faecalis purity was carried out through gram staining and evaluation of colony morphology post-cultivation. After the 30-day period [17], a 100 µL portion of broth from samples without leakage was subcultured onto blood agar plates to confirm the absence of leakage. The microbiologist was blinded to the group assignments during the microbiological procedures and the evaluation of microbial leakage.

Statistical Analysis

Descriptive statistics were presented as frequencies and percentages for qualitative variables (such as positive bacterial growth). For quantitative variables (including time to bacterial growth), means, standard deviation (SD), medians, and interquartile range (IQR) were calculated. The comparison of positive bacterial growth utilized the chi-squared test with Monte Carlo correction of the p-values. The Kruskal-Wallis test was employed for comparing time to bacterial growth, followed by multiple pairwise comparisons using a Bonferroni-adjusted significance level. Statistical significance was established at a p-value < 0.05. The data analysis was performed using IBM SPSS for Windows (Version 26.0).

RESULTS

Throughout the entire experiment, the negative control samples consistently maintained an

uncontaminated condition. The results of positive bacterial growth in the study groups after 30 days of E. faecalis inoculation are presented in (Table 1 and Figure 2), while (Table 2 and Figure 3) display the time to bacterial growth in these groups. None of the test groups completely resisted bacterial microleakage, with such leakage predominantly occurring in the latter half of the experimental period. Notably, only one sample (10%) in the Sole apicectomy group (group1) exhibited positive bacterial growth, indicated by broth turbidity on day 26. In the standard retrograde procedure group (group2), two samples (20%) showed turbidity after an average time of 18 days (at day 15 and day 26). Post hoc comparison revealed no statistically significant difference between these two test groups.

Figure 1: Experimental Apparatus.

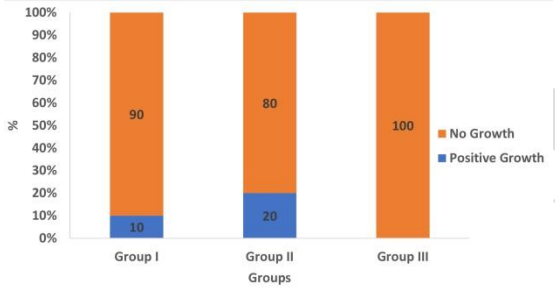

Figure 2: Positive bacterial growth in the study groups.

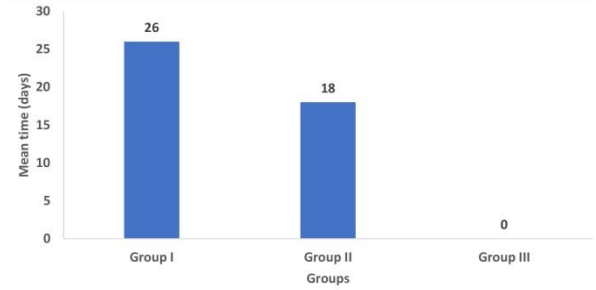

Figure 3: Time to positive bacterial growth in the study groups.

Table 1: Positive bacterial growth in the study groups

Positive growth	Group I (n=10)	Group II (n=10)	Group III (n=10)	X^2 (p value)
	N (%)			
Yes	1 (10%)	2 (20%)	0 (0%)	X^2= 3.96
No	9 (90%)	8 (80%)	10 (100%)	P_{MC}= 0.27

X^2: Chi-squared test, P_{MC}: Monte Carlo corrected p value
*statistically significant at p value <0.05

Table 2: Time to bacterial growth in the study groups

	Group I (n= 1)	Group II (n=2)	Group III (n= 0)	KWT P value
	N (%)			
Mean (SD)	26.00 (0.00) **a**	18.00 (11.31) **a**	0.00 (0.00) **b**	Z= 11.86
Median (IQR)	26.00 (0.00)	18.00 (16.00)	0.00 (0.00)	**P= 0.003***

KWT: Kruskal Wallis test, SD: Standard Deviation, IQR: Interquartile Range
*statistically significant at p value <0.05
a.b: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level

DISCUSSION

The primary objective of root-end management in surgical endodontic procedures is to stop bacteria and their resulting products from the root canal system from entering the surrounding periradicular tissues. If the retrograde filling material effectively seals the root, it prevents bacterial biofilms, toxins, and byproducts from entering periapical tissues, thereby avoiding irritation and promoting healing [25].

The ultimate outcome of endodontic surgery depends on various clinical factors, including effective hemorrhage control, proper access, assessment of periodontal disease extent, appropriate preparation for retrograde procedures, correct retrograde material utilization, consideration of occlusal trauma, identification of missed vertical fractures and isthmuses, evaluation of orthograde filling quality, and understanding individual host responses. Not all teeth may be suitable for standard retrograde ultrasonic preparation and retrograde obturation. [26]. Additionally, there is a risk of chipping, cracks, and fractures during root-end cavity preparation [27]. Therefore, our experimental design aims to investigate bacterial microleakage following root end resection, employing orthograde obturation with a single cone technique and bioceramic sealer as a potential and practical alternative technique, omitting retrograde obturation.

The current research employed the bacterial microleakage test. A clear benefit of this approach is its enhanced clinical relevance, as it does not involve the use of reactive chemicals or radiation. Nevertheless, there are certain limitations associated with this method. It relies on the basic assumption that leakage solely occurs through the root canal space and not through other potential pathways [3].

To tackle this issue, the present investigation implemented thorough steps to ensure that the passage of bacterial microleakage occurred exclusively through the tooth apex into the suspension broth. This was achieved by applying dual coats of nail polish to the outer surfaces of the roots. Furthermore, meticulous sealing was performed on all connections within the assembled apparatus, involving the sample, tube, and vial, using cyanoacrylate, followed by an additional layer of nail varnish [17,21]. The effectiveness of this assembly was demonstrated by the absence of BHI turbidity in all negative controls over a 30-day period, indicating successful sealing between the different components of the apparatus.

Ensuring nutritional replenishment in the system is essential to sustain the vitality of bacteria throughout the 30-day experiment. The decision to use the E. faecalis strain in this research was influenced by several factors. Gram-positive and facultative anaerobes, particularly E. faecalis, are commonly found in root canal-treated teeth with persistent infections. E. faecalis is notable for its capability to form a biofilm, enhancing its resistance to destruction. Additionally, it is recognized for being highly resistant, able to endure alkaline environments and glucose deprivation [28–30].

In this research, NeoMTA® 2 (NEO; Avalon Biomed Inc.) and NeoSealer Flo were selected because they share similar components, both being products developed by the same company.

A recent investigation revealed that Neo MTA Plus triggers increased formation of mineralized nodules and results in greater and more extended release of calcium and hydroxyl ions compared to MTA Angelus [31]. In a prior study, Neo MTA Plus was suggested as the preferred substance for root-end filling due to its minimal microleakage, distinguishing it from other materials such as Pro Root MTA, BIODENTINE, and Glass Ionomer Cement [14]. Conversely, Neo MTA Plus boasts a quick setting time and resistance to washout.

In the current findings, both techniques demonstrated effective sealing capabilities against bacterial microleakage. In the sole apicectomy group, only one sample (10%) exhibited microleakage, while in the standard retrograde procedure group, two samples (20%) showed microleakage, with no statistically significant difference between the two techniques. Therefore, based on the current results, the null hypothesis is fully accepted.

Challenges related to difficult accessibility, anatomical intricacies, procedure duration, and clinician expertise will not pose significant issues in the sole apicectomy technique. On the contrary, the process of root resection, particularly following orthograde filling with the SCT and bioceramic sealer, is easily executed with reduced technical sensitivity and enhanced handling characteristics. This ease of application not only saves time during the procedure but also has the potential to elevate the success rate of apical surgeries. Additionally, the pre-mixed nature of bioceramic sealer contrasts with the manual mixing required for MTA, potentially affecting the consistency of the mixture.

One possible application of the aforementioned technique is intentional replantation. Prior studies have underscored the significance of the time the tooth spends outside the mouth for the success of this procedure [32]. Limiting the procedure to root resection alone could potentially expedite the process and improve the overall success rate of intentional replantation.

The standard retrograde procedure, using MTA as a retrograde filling, served as the benchmark in this study. MTA, recognized for its biocompatibility and consistent outcomes in endodontic surgery, has been associated with benefits such as the absence of inflammation, cementum deposition, and the formation of hard tissue [33]. Despite these positive biological properties, MTA has certain limitations. Its granular consistency makes it somewhat challenging to handle and to be applied in the prepared retrocavities during endodontic surgical procedures [34,35]. This could be the explanation of the higher incidence of turbidity with MTA.

Interestingly, the sole apicectomy group demonstrated effective sealing capabilities. This can be attributed to the bioceramic sealer's improved flow, enabling superior penetration of dentinal tubules and optimal adaptation of the sealer in the critical apical region. This adaptation helps prevent bacterial recolonization. Additionally, the bioceramic sealer's ability to generate calcium hydroxide may contribute to the formation of hydroxyapatite when comes in contact with body fluids which can represent the main reason for formation of a direct bond between the dentin and the sealer [36].

In a previous study, the effectiveness of apicectomy alone was evaluated using micro-CT to quantify void volumes. The results showed fewer voids compared to the traditional method that utilizes MTA for retrograde filling, which is consistent with our own findings [37]. However, other research studies have indicated that obtaining a reliable apical seal is difficult without root end filling [38,39]. These studies reported a notable rate of failure when surgical endodontic treatment,

involving apicectomy without retrograde filling, was initially introduced.

While retrograde filling is believed to offer protection against potential leakage resulting from lingering bacterial infections, it appears not to considerably enhance the outlook when compared to root canals featuring a dense orthograde filling [37]. Earlier research has indicated that bacterial biofilm can still inhabit and infiltrate deep into dentinal tubules, even when root end filling is present [3,40].

The current study demonstrates strength in employing microbial leakage equipment to assess bacterial microleakage following root end resection, specifically after the use of SCT and bioceramic sealer for the first time. However, certain limitations should be acknowledged. The study is confined to an in vitro setting, and factors such as the chosen experimental model, the relatively short observation period, absence of clinical conditions like pH variations, saliva presence, periapical lesions, bleeding control, and clinician expertise may impact the findings. Additionally, it is crucial to note that the application of SCT with bioceramic sealer is feasible only in cases where orthograde RCT is possible; its use is restricted when a retrograde approach becomes necessary.

Nevertheless, the research lays the groundwork for additional clinical investigations aimed at establishing uniform guidelines for validating the in vitro findings of this technique. Subsequent research is essential to evaluate microleakage over extended durations, ensuring more dependable outcomes. Moreover, other obturation techniques such as warm vertical and cold lateral compactions could be included in comparison with the SCT.

CONCLUSION

According to the results of this in vitro study, it is suggested that performing root end resection following a single cone technique obturation, coupled with a bioceramic sealer without retrograde obturation, might provide a potentially promising and simplified method for root-end surgery. This could be regarded as an alternative to the well-established gold standard for endodontic apical surgery, as noted within the limitations of the current study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING STATEMENT

The authors received no specific funding for this work.

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