ASSESSMENT OF ANTIBACTERIAL EFFECT AND FLOWABILITY OF BIOCERAMIC SEALER MODIFIED WITH $BaTiO_3$ NANOPARTICLES

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ABSTRACT

Introduction: One of the main causes of endodontic treatment failures is the persistence of microorganisms within the root canal systems. Piezoelectric materials, including barium titanate ($BaTiO_3$), offer antibacterial effects. The aim of this project is to develop an endodontic sealer embedded with piezoelectric fillers for the prevention of root canal infections.

Materials and methods: $BaTiO_3$ particles were mixed with EndoSequence (BC) sealer in two concentrations (5% and 10%wt). Flowability test was conducted for each type of sealer according to ISO-6876 guidelines. The antibacterial evaluation was performed using an ex-vivo model. Single-rooted extracted teeth were instrumented, and canals were infected with *Enterococcus faecalis* for 7 days. Following the root canal treatment, the sealers (BC, BC+ $BaTiO_3$-5%, BC+ $BaTiO_3$-10%) were used for obturation. Untreated teeth were used as positive control. Specimens with $BaTiO_3$ particles were subjected to compression cyclic loading to activate the piezoelectric charges and resemble mastication forces. Cell viability (CFU/mL) was used to determine the number of bacteria at the bonded interface of the sealant. ANOVA was used to evaluate the statistical differences among the groups.

Results: The addition of $BaTiO_3$ particles into BC Sealer resulted in a decrease in flowability (BC: 21.7 ± 0.55 mm, BC+ $BaTiO_3$-5%:19.5 ± 0.50 mm, BC+ $BaTiO_3$-10%:17.44 ± 0.40 mm). All sealers exhibited antibacterial properties. The addition of $BaTiO_3$ nanoparticles significantly enhanced the antibacterial efficacy compared to BC

sealer. However, there was no significant difference between the BC+BTO 5% and BC+BTO 10% groups (BC: 3.90 ± 0.27, while both the BC+BTO 5%: 3.31 ± 0.12, BC+BTO 10%: 3.01 ± 0.22).

Conclusion: An antibacterial piezoelectric endodontic sealer was developed. Adding more than 5%w of $BaTiO_3$ particles into BC sealers enhanced the antimicrobial efficacy. However, adding more than 10% of $BaTiO_3$ negatively affects the sealer's flow properties.

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CHAPTER 1

INTRODUCTION

1.1 Background

The invasion of bacteria and their byproducts into the root canal system is the primary cause of pulpal and periapical disease. Microbial infection affecting the dental pulp has been known as the primary cause of endodontic diseases which need endodontic treatment [1]. Kakehashi et al.'s experiment on germ-free rats proved that bacteria is necessary to initiate and progress pulpal disease to apical periodontitis. Their study showed that genetically modified germ-free rats did not develop apical periodontitis following pulpal exposure. In contrast, the progression of the pulpal disease to apical periodontitis was seen in rats with normal oral flora [2].

The main objectives of root canal therapy include the elimination of diseased pulp tissue, the cleaning and shaping the root canal system, disinfection of the contaminated canals, and filling the root canal system to prevent further infection [1]. Previous studies indicated that nonsurgical root canal therapy offers patients a reliable method for preserving diseased teeth. However, the success rate of root canal treatments is not 100% [3]. Several factors may influence reported outcomes including pre-existing dental conditions such as bacteria and preoperative periapical lesions, intertreatment factors such as level of root filling, and posttreatment coronal leakage and restorative care. Lin et al., suggested that the main contributing factors to endodontic failures are the persistence of bacterial infection in the canal space and/or the peri-radicular area and the presence of preoperative peri-radicular rarefaction [4]. Sjogren proposed that the apical level of the root filling can significantly affect the treatment outcomes in teeth with

necrotic pulps and periapical lesions [5]. Several retrospective studies indicate that most teeth removed following root canal therapy are not properly restored. Salehrabi and Rotstein found that, of those teeth extracted following root canal therapy, 85% did not have a definitive coronal restoration [6]. Advancements in biomedical technologies and novel antibacterial biomaterials may improve the success rate of endodontic treatment by minimizing the risk of infection and the likelihood of failure.

1.2 Endodontic Infection and *Enterococcus Faecalis* (*E. faecalis*)

Microorganisms are the primary driver of endodontic infections. These infections differ from other oral infections because they progress in an isolated and enclosed system surrounded by hard tissues known as the root canal system. After the initiation of the disease, when the bacteria enter the coronal tooth structure through a carious lesion or a traumatic injury, they are limited to the intra-radicular region. Eventually, if the condition is not resolved, the microbes and their by-products exit the apical foramen and spread to the peri-radicular tissues [7,8].

Endodontic infections are highly complex microbial communities. Ordinola-Zapata showed the community of microorganisms of endodontic infections using 16S rRNA next-generation sequencing analysis (NGS)[9]. Nine microbial genera comprised the predominant taxa, including Parvimonas, Fusobacterium, Campylobacter, Arachnia, Eubacterium, Prevotella, Peptostreptococcus, Fretibacterium, and Pseudoramibacter [9]

Siqueira et al. discovered the presence of several microbial species in failed endodontic treatments using the polymerase chain reaction (PCR) [10]. They found that *E. faecalis* was the most prevalent species existing in 77% of the failed root canal treatments [10]. *E. faecalis*, a facultative anaerobic gram-positive coccus, is the most

common Enterococcus species found in failed and secondary endodontic infections. Stevens et al. found that this bacterium is even resistant to calcium hydroxide, the most common antibacterial intra-canal medicament used in endodontic treatments [11]. Eliminating *E. faecalis* is easier in the primary stages of the disease when they are present in small quantities. Once this bacterium is established in the dentinal tubules, eradicating it becomes very challenging. *E. faecalis* cells can outgrowth, remove other microbes and can thrive in the low-nutrient environment of the treated canal [12,13].

1.3 Endodontic Sealers

The primary goal of endodontic treatment is to save an infected tooth by eliminating or minimizing the load of pathogens to the level that allows the host immune system to contribute to the healing process of pulpal and peri-radicular infections. Due to the complex pulpal anatomy and the limitations of cleaning and shaping methods, achieving this goal remains challenging. Even with contemporary endodontic advancements, the success rate is 74% to 88% [14].

During root canal treatment, endodontic sealers are essential for filling the space between the root canal wall and gutta-percha cones. An ideal endodontic sealer should have high radiopacity, excellent sealing ability, low solubility, appropriate viscosity and setting time, antibacterial properties, non-cytotoxicity, and strong adhesion to the dentinal wall [15]. Endodontic sealers are generally categorized into five main groups depending on their setting reaction and composition: zinc oxide eugenol, salicylate, fatty acid, glass ionomer, silicone, epoxy resin, tricalcium silicate (MTA/bioceramic), and methacrylate resin [15,16].

Endodontic sealers offering antimicrobial effects may improve the success rate of endodontic treatment. Endodontic sealers available in the market (Table 1) often offer some antimicrobial properties. For example, Sealapex, a calcium hydroxide-based sealer, is antimicrobial due to its high Ca^{2+} content [17]. Mineral Trioxide Aggregate (MTA) consists of tricalcium silicate, aluminate, silicate oxide, and bismuth. It has been compared to Portland cement due to its chemical composition and tissue response similarities. It has also demonstrated antibacterial activity because of its high pH in the microenvironment. Zinc oxide-eugenol sealers, one of the oldest available products, are renowned for their antibacterial activity against facultative microorganisms due to the presence of eugenol. This sealer contains zinc oxide, hydrogenated resin, barium sulfate, anhydrous sodium borate, eugenol, and almond oil. Current endodontic sealers have demonstrated limited clinical effectiveness in terms of antibacterial properties since their antibacterial effects tend to decrease over time after they are set [17].

Table 1. Different Types of Endodontic Sealers

Type of Endodontic Sealer	Brand Name
Zinc Oxide Eugenol-Based	Pulp Canal Sealer, Roths'sealer, Tubliseal
Calcium Hydroxide-Based	Sealapex, Apexit Plus
Resin-Based	AH Plus, Epiphany
Silicone-Based	GuttaFlow, RoekoSeal
MTA-Based	MTA Fillapex, Endo CPM Sealer
Glass Ionomer-Based	Ketac-Endo, Ionoseal

1.4 Bioceramic Endodontic Sealers

Bioceramic sealers are biomaterials based on calcium silicate. They exhibit excellent sealing ability and can set in the presence of moisture. Their positive interactions with biological tissues are mainly attributed to the release of biologically active ions and the

formation of an apatite layer on their surface. These properties make calcium silicate-based materials an excellent choice for managing complex endodontic cases such as perforation repair and apical plugs for teeth with open apexes [18].

Endosequence BC Sealer (Brasseler USA, Savannah, GA) is one of the most popular bioceramic sealers. This sealer contains zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, and thickening agents. Due to its highly alkaline pH, this sealer also possesses antibacterial properties during the setting reaction. While new bioceramic sealers possess potent antibacterial activity, they have not eradicated *E. faecalis* [19].

1.5 Ex-vivo Models to Evaluate the Antibacterial Effects of Endodontic Sealers

Previous studies have suggested various methods for assessing the antibacterial properties of dental materials. The agar diffusion test (ADT) is commonly used as the standard assay despite its limitations [21]. These limitations include its semiquantitative nature, inability to measure the activity of soluble components, failure to distinguish between bacteriostatic and bactericidal effects, and the challenges in controlling many variables. The accurate performance of the ADT necessitates precise standardization of several factors, including inoculum density, medium composition, agar viscosity, storage conditions for agar plates, size and quantity of specimens per plate, positioning and layout of specimens on a plate, spacing between specimens and adjacent agar, as well as incubation time and temperature [20]. The direct contact test (DCT), introduced by Weiss et al., is another standard method used to evaluate the antibacterial properties of dental materials [21]. This test measures the impact of direct and close contact between the test microorganism and the tested material on microbial viability, regardless of the solubility

and diffusability of the antimicrobial components. This method provides a more reliable and reproducible quantitative test for demonstrating the activity of insoluble antibacterial components [21].

Dentin plays a crucial role in the endodontic environment due to its unique properties, which can influence the antimicrobial effects of endodontic biomaterials. The intricate network of dentinal tubules can facilitate communication between the pulp and the external environment and harbor bacteria and their by-products in infected teeth. Several studies have suggested that dentin can inhibit the antimicrobial activity of endodontic agents by acting as a physical barrier and preventing the penetration and diffusion of antimicrobial agents [22]. To better assess the antimicrobial efficacy of new endodontic biomaterials, utilizing extracted tooth models that incorporate dentin substrates is advantageous. These models more accurately replicate clinical conditions by accounting for the potential influence of dentin, thus aiding in developing strategies to enhance the effectiveness of endodontic sealers in the presence of dentin [22,23].

1.6 Flowability of Endodontic Sealers

The proper flow of endodontic sealers is crucial for effectively filling accessory canals and voids between gutta-percha cones and dentinal walls. Sufficient flow ensures the sealer can reach and fill irregularities within the root canal system. However, excessive flow can lead to apical extrusion, potentially damaging the periapical tissues. Striking a balance is essential to maximize their effectiveness while minimizing adverse effects on surrounding tissues [24,25].

The International Organization for Standardization (ISO) is a global organization that develops international standards for evaluating the properties different materials. These

standards assess characteristics and establish criteria for suitability in clinical settings. ISO-6876 specifies the requirements and testing procedures for endodontic sealers. One evaluation includes the flowability of endodontic sealers. The criteria stands that a minimum 17 mm disc diameter is necessary for adequate clinical flowability.

1.7 Piezoelectric materials

Piezoelectric biomaterials are a special class of materials that produce electrical charges upon mechanical stimulation. For example, when subjected to external stress and deformation, such as bending or stretching, these materials undergo a change in their internal structure, leading to the redistribution of electric charges and the generation of electric potential. Recently, piezoelectric materials like $BaTiO_3$ have been shown excellent biocompatibility and use in the medical and dental fields. Specifically, piezoelectric materials have proven different therapeutic effects including bone regeneration, drug delivery, and tissue engineering [26,27]. Recently, piezoelectric materials have demonstrated promising antibacterial effects against different oral pathogens [28]. The produced electrical charges can interfere with bacterial cell membranes, disrupt cellular processes, and ultimately cause bacterial death [27,29].

Incorporating bioactive fillers or particles into endodontic sealers is a topic of interest due to its potential to enhance antimicrobial activity and improve treatment outcomes. Kishen et al. [23] were the first to investigate the impact of cationic nanoparticles on root canal disinfection. Their findings revealed that treating dentin with cationic nanoparticles led to reduced adherence of *E. faecalis*. Subsequently, numerous studies have explored the antibacterial properties and functional applications of nanoparticles in endodontics for root canal systems [29]. Adding piezoelectric particles to endodontic sealers may increase the

chances of success of root canal treatments. Limited information exists whether $BaTiO_3$ particles have antibacterial effects against endodontic pathogens. This study investigates the potential advantages of integrating $BaTiO_3$ as an antibacterial agent in calcium silicate-based endodontic sealers.

CHAPTER 2

AIMS AND OBJECTIVES

This study aims to develop an endodontic calcium silicate-based sealer incorporated with barium titanate ($BaTiO_3$) nanoparticles for enhanced antibacterial effects. The specific objectives of this study are:

1. To assess the antibacterial effects of $BaTiO_3$-incorporated calcium silicate sealers ex-vivo.
2. To evaluate the flowability of calcium silicate sealers incorporated with $BaTiO_3$ according to the ISO-6876 standard.

CHAPTER 3

RESEARCH SIGNIFICANCE

Piezoelectric materials have shown promising antibacterial effects. However, there is limited research on integrating $BaTiO_3$ piezoelectric particles into endodontic sealers for enhanced antibacterial efficacy. With the increasing demand for antibacterial and biocompatible sealers among clinicians to improve the clinical longevity of root canal treatment, it would be highly beneficial to explore whether adding $BaTiO_3$ piezoelectric particles can improve the antimicrobial efficacy of calcium silicate sealers. Furthermore, it is critical to establish the optimum concentration of these particles in the bioceramic sealer to ensure the proper flow properties of the material for successful clinical application. Investigating the potential applications of $BaTiO_3$ piezoelectric nanoparticles in endodontic sealers through this research will enhance our understanding of the capabilities of these materials and may improve the effectiveness of root canal treatments.

CHAPTER 4

MATERIALS AND METHODS

4.1 Sealer Preparation:

The sealer samples were prepared using a calcium silicate-based bioceramic sealer (EndoSequence BC Sealer, Brasseler, Savannah, GA). Barium Titanate ($BaTiO_3$) with a size of 200 nm (US. Nano) were added to the BC sealer in different proportions including 5% w/v and 10% w/v. BC sealer and piezoelectric nanoparticles were mixed in a planetary mixer at 2,000 rpm for 2 minutes. BC sealer without $BaTiO_3$ was used as a control group.

4.2 Flow Test:

The flow test was carried out following the instructions in the ISO 6876. Briefly, a drop of 0.05 mL of sealer was placed in the center of a glass slab and left undisturbed for 3 minutes. Then, a glass and a weight totaling 120 g were carefully placed on top of the droplet and left for 7 minutes. The longest and shortest diameters were measured with a high-precision caliper, and the mean value was recorded. Each group was measured using three samples (N=3). Three groups were evaluated: BC (group 1), BC + BTO (5% wt, group 2), and BC + BTO (10% wt, group 3). This experiment is illustrated in Figure 1.

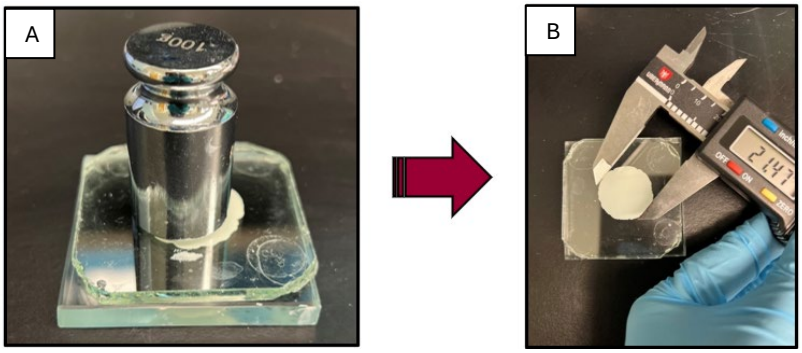

Figure 1. (A) A glass and a weight totaling 120g on top of the sealer droplet. (B) The diameters of the sealer samples were measured using a caliper.

4.3 Ex-vivo Model to Evaluate the Antibacterial Effects:

An ex-vivo model was used to evaluate the antibacterial effects of sealers [22]. Single-rooted extracted teeth were collected and maintained in Hank Balanced Salt Solution (HBSS) at 2°C. The crowns were dissected using a sterile high-speed handpiece and bur. The working length was measured using a #10K file (Dentsply Sirona), and the canal was instrumented with a ProTaper Gold file system (Dentsply Sirona) up to size F4 (40/0.65). Canals were irrigated with 2.5 % sodium hypochlorite followed by 17% EDTA. The apical 2mm of each root was cut. All specimens were standardized to a length of 10 mm. Samples were sanitized by ethanol rinse and UV light for 24 hours. Steps are illustrated in Figure 2.

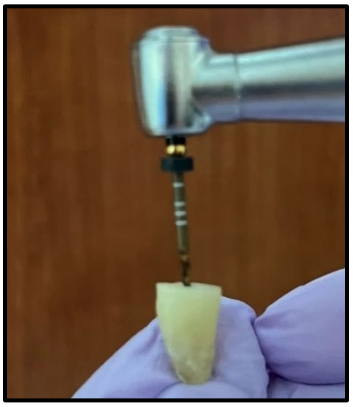

Figure 2. Teeth were decoronated, and canals instrumented to size F4.

4.3.1 Bacterial Inoculation

We inoculated the *E. faecalis* (American Type Culture Collection ATCC 29.212) in BHI (Brain Heart Infusion) medium for 24 hours. A colony was harvested and used to prepare a liquid culture of *E. faecalis* in BHI media. The optical density was adjusted to OD_{600}=0.1 at 600 nm which is equivalent to 8×10^8 cells/mL, after the culture was incubated at 37°C for additional 24 hours.

To infect teeth, the tooth prepared samples were submerged in separate wells with the BHI media for 1 week. Media was refreshed every 48 hours. After incubation, the tooth

samples were rinsed with phosphate-buffered saline (PBS) to eliminate unattached or deceased bacteria (see Figure 3).

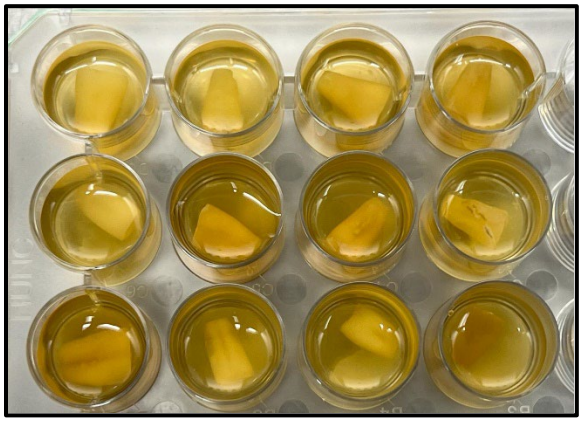

Figure 3. Root samples in *E. Faecalis* inoculate.

4.3.2 Samples Obturation

After infection, the root canals were prepared for obturation with sealers. Samples were dried using sterile paper points before being divided into four groups based on the sealer. The samples were divided into 5 groups based on the root canal sealer used. The canals were filled with gutta-percha and BC sealer (group 1), BC sealer and $BaTiO_3$ (5% wt) (group 2), BC sealer and $BaTiO_3$ (10% wt) (group 3), uninfected tooth without sealer as the negative control (group 4) and infected tooth without sealer as the positive control (group 5). Each canal was obturated with size F4 gutta percha using single cone technique. All procedures were carried out in a biosafety cabinet to prevent potential cross-contamination. Figure 4 shows an example of how the tooth were obturated with sealers.

Figure 4. Specimens were obturated with sealers.

4.3.3 Mastication – Repetitive Forces During Cell Culture

To activate the antibacterial effects in the piezoelectric sealants, samples need mechanical stimulation resembling mastication forces. The specimens were secured in an acrylic fixture using putty and then subjected to cyclic compression loading using a piezoelectric activator (Mechano Culture). A load of 22 MPa and a frequency of 2 Hz were applied to the samples to simulate mastication forces for 24 hours (see Figure 5).

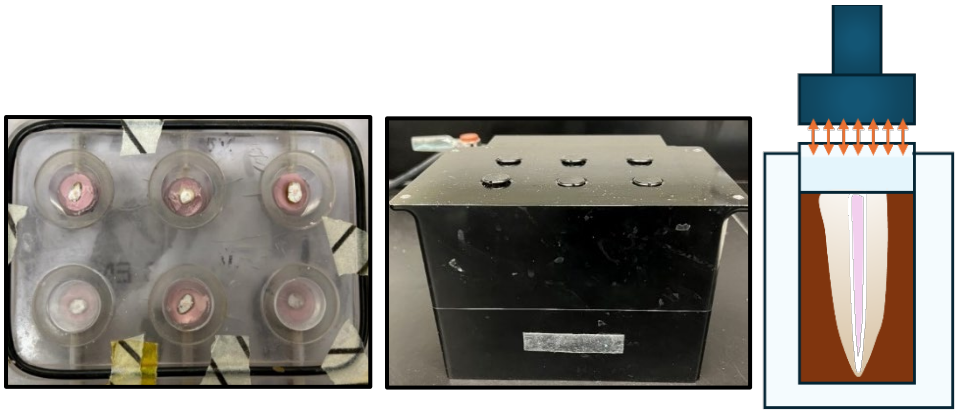

Figure 5. Specimens were secured in an acrylic fixture and subjected to mechanical force using a piezoelectric activator.

4.4 Antibacterial Evaluations and Cell Viability (CFU):

After incubation, all roots were longitudinally sectioned into two halves using a thin diamond bur with a dental high-speed handpiece. One half was used for microbiological analysis (i.e., CFU count), while the other half was used for microscopic analysis (Live/Dead fluorescent microscopy). To determine the antibacterial effects, dentinal shavings were collected at the interface between the tooth/sealer utilizing the full depth of a round bur (ISO size #4 diameter =1.4 mm) along the root canals (Figure 6). The dentin powder was collected in microcentrifuge test tubes containing 1 mL BHI solution, vortexed, serially diluted three times, and plated on a BHI agar plate. The plates were incubated at 37°C for 24 hours. After the incubation period, colonies of viable bacteria were counted, and they were transferred to $\log_{10}$ to calculate the colony-forming units (CFU/mL).

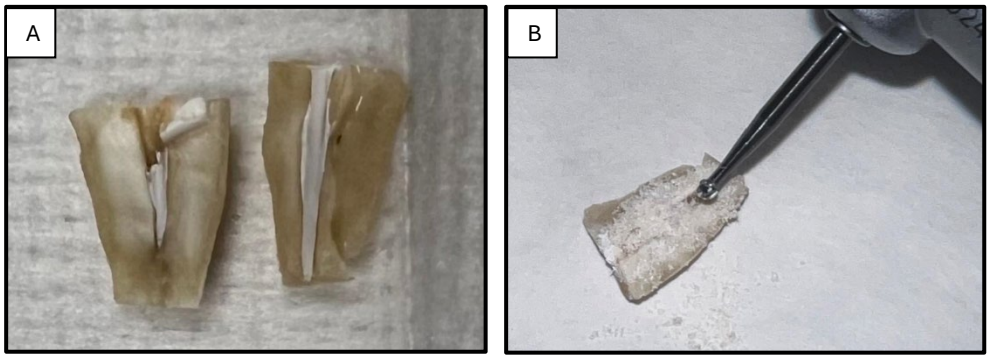

Figure 6. The specimens were sectioned (A), and dentinal debris was collected to perform the antibacterial evaluation (B).

4.5 Live Cells Fluorescence Microscopy:

Live and dead bacteria were visualized utilizing fluorescence microscopy (EVOS M5000). Bacterial cells were stained with a LIVE/DEAD BacLight bacterial viability kit (ThermoFisher Scientific). According to the manufacturer's instructions, a fluorescent

stain solution was prepared by mixing 3 μL of SYTO9 and 3 μL of propidium iodide (PI) in 1 mL of ultrapure water. Each specimen's surface was stained using 200 μL of the staining solution and incubated in a dark room for 20 minutes. The excess dye was removed from the specimens' surfaces by rinsing them with filter-sterilized water. Fluorescence images of the biofilms were captured as a series of 50 z-stacks with identical settings, and intensities were adjusted for background fluorescence. The color images were converted to grayscale and thresholded to quantify live and dead bacteria to produce black-and-white images. Pixels above the threshold (white pixels) were counted separately for live and dead images.

4.6 Statistical Analysis:

This study was conducted by using five samples per group. All data are presented as mean ± standard deviation. Statistical differences were assessed using a one-way ANOVA with a significance level of 0.05.

CHAPTER 5

RESULTS

5.1 Flow Test

This study found that the Endosequence BC sealer exhibited an average diameter of 21.7 ± 0.55 mm, showing the product's compliance with the guidelines outlined by ISO 6786 (see Figure 8). Incorporating barium titanate particles into the BC sealer at 5% wt resulted in a decrease in flow to 19.5 ± 0.50 mm. Increasing the barium titanate nanoparticles into the BC sealer at 10% wt resulted in the most significant reduction in the flowability to 17.44 ± 0.40 mm. However, these findings indicate that adding 5% and 10% of BTO to BC sealer complies with ISO 6786 standards. However, adding more than 10% of BTO may negatively affect the flow properties of the piezoelectric sealer.

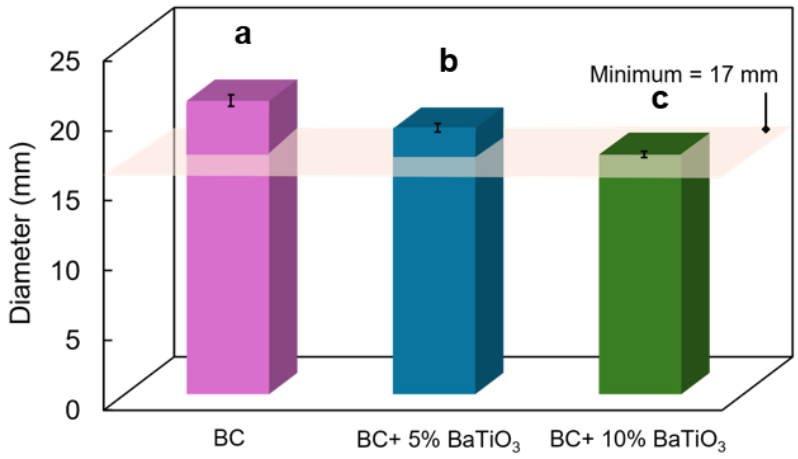

Figure 7. The flow f BC and barium titanate-incorporated sealers. The mean and standard deviation (N=3). Different letters represent a significant difference between groups ($p<0.05$). The horizontal line indicates the minimum flow recommended by ISO 6786 guidelines.

5.2 Antibacterial Evaluations. Cell Viability (CFU):

The colony-forming unit (CFU) test was conducted to quantify the number of viable bacteria in the samples treated with different endodontic sealers (Figure 8). The results are expressed as log(CFU) and summarized in Figure 9. The CFU counts for each treatment group were as follows: The positive control group (lacking sealer) displayed the highest count of viable cells among the groups with a log (CFU) of 4.60 ± 0.25. All other groups, including BC, BC+BTO 5%, and BC+BTO 10%, exhibited a significant reduction in colony-forming units (CFUs) compared to the positive control. The specimens treated with BC exhibited a log(CFU) count of 3.90 ± 0.27, while the BC+BTO 5% and BC+BTO 10% exhibited a CFU of 3.31 ± 0.12 and 3.01 ± 0.22 respectively. These findings indicate that all tested endodontic sealers possess antimicrobial properties, with BC+BTO 10% showing the highest efficacy in reducing bacterial viability. However, no significant difference was noted between BC+BTO 5% and BC+BTO 10% groups.

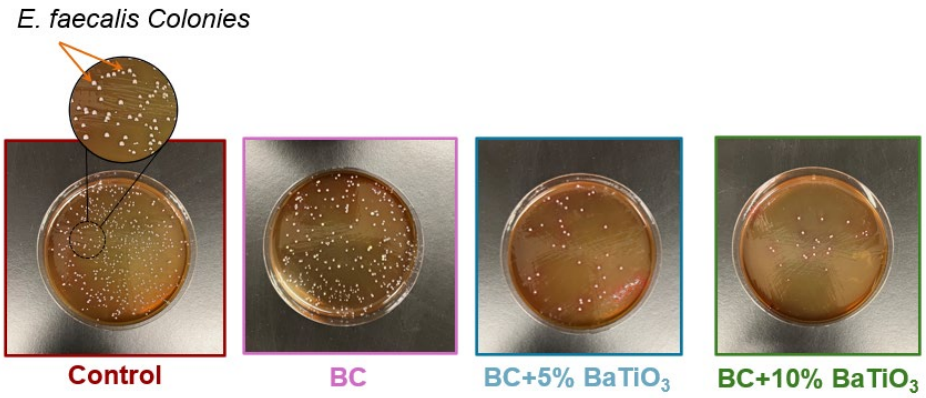

Figure 8. Blood agar plate with colonies of *E. faecalis*

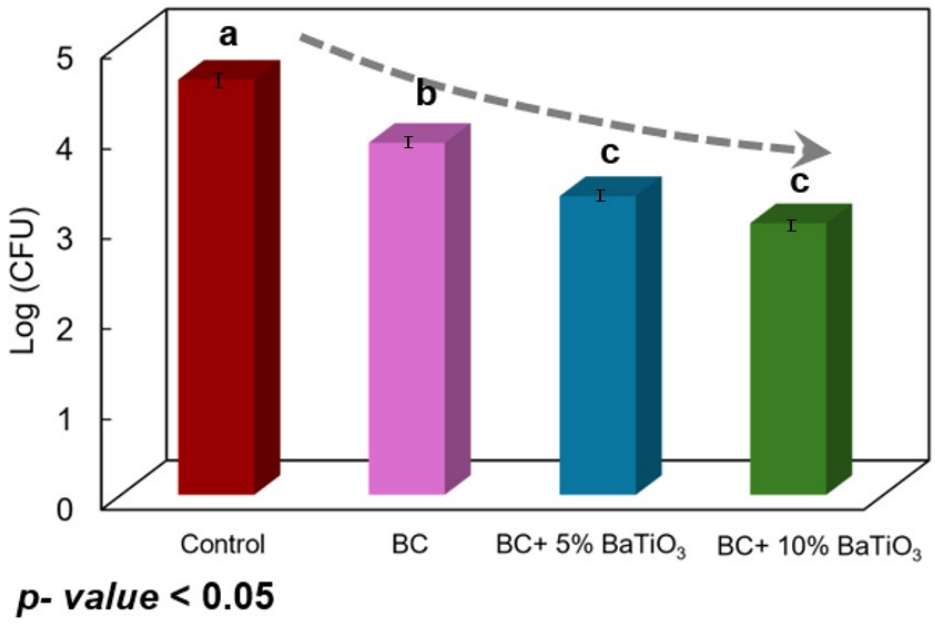

Figure 9. Addition of $BaTiO_3$ significantly reduced the number of viable cells compared with the BC sealer and positive control. No significant difference was seen between the 5% $BaTiO_3$ and 10% $BaTiO_3$ groups.

5.3 Live Cell Fluorescence Microscopy

Live cells were visualized in samples treated with different endodontic sealers. Overall, the results obtained from fluorescent microscopy observations were consistent with the CFU test results, reinforcing the sealers' efficacy in reducing bacterial viability. The control group (BC) exhibited a high proportion of live bacteria noted by higher quantity of green color. Treatment with BC+ $BaTiO_3$ resulted in a significant reduction in live bacterial viability (less green color). Treatment with both BC+ $BaTiO_3$ 5% and BC+ $BaTiO_3$10% were comparable. Figure 10 displays a fluorescent images showing the live cells attached to the interface.

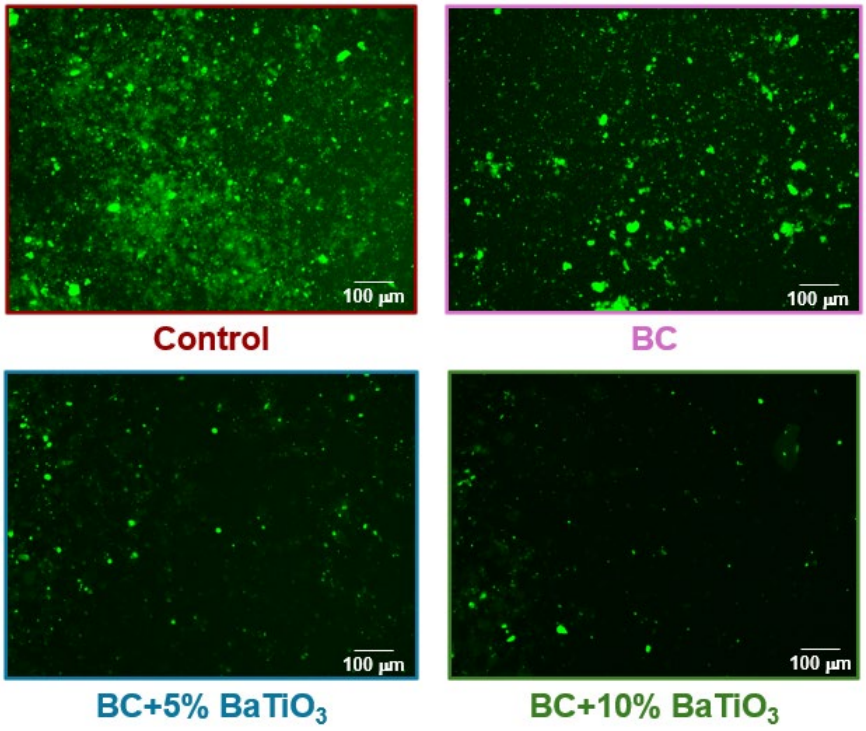

Figure 10. Fluorescent image showing live cells stained with green.

CHAPTER 6

DISCUSSION

6.1 Flowability of the Sealers

Flowability tests were performed on three sealers: BC, BC+ $BaTiO_3$ 5%, and BC+ $BaTiO_3$ 10%, according to the ISO 6876 test method. The proper flow of endodontic sealers is essential for effectively filling accessory canals and voids between gutta-percha cones and dentinal walls. Ensuring sufficient flow allows the sealer to reach and fill irregularities within the root canal system. Our results show that BC has the highest flow ability, and as the concentration of the $BaTiO_3$ increased, the flow rate decreased. The reduced flow rate can be attributed to the increased viscosity caused by adding $BaTiO_3$ particles. Higher viscosity negatively affects the sealer's flow characteristics, making it more resistant to moving and filling the canal irregularities. This can be an undesirable effect on the BC sealer. Also, the particle size of endodontic sealers significantly affects the sealer's flowability and viscosity. Generally, an increase in particle size leads to a decrease in the flow rate of sealers. The higher viscosity is due to the decreased space between particles, resulting in more interparticle interactions that impair the sealer's ability to flow smoothly. Larger particles have more surface area. The increase in the surface area increases interparticle friction, creating more resistance to movement and reducing flow. Moreover, larger particles contribute to higher surface tension, as the cohesive forces among liquid molecules surrounding the particles become stronger, restricting their movement. This combination of factors results in endodontic sealers with reduced flowability, impacting their effectiveness in clinical applications.

6.2 Antibacterial Effects of Modified Sealers

In this research, we found that piezoelectric particles can enhance the antibacterial properties of endodontic sealers. Specifically, we showed that these charges have the potential to effectively eradicate *E. faecalis*, thus contributing to the increase of root canal treatment longevity. Our findings align with the results of the previous similar studies [28,29]. For instance, we observed a significant reduction in pathogenic bacteria due to the application of piezoelectric charges, which aligns with the results reported by Montoya et al. (2021), who demonstrated similar antimicrobial effects against Streptococcus mutans using $BaTiO_3$ piezoelectric materials [29]. Previous research shows these piezoelectric biomaterials present several advantages, such as biocompatibility, low cytotoxicity, and long-term therapeutic effects. Unlike current antibacterial materials, which primarily provide short-lived antibacterial effects due to the depletion of the antibacterial agents, piezoelectric biomaterials generate continuous electrical charges for over 12 million cycles of mechanical loading and unloading. This durability equates to approximately 24 years of clinical service, based on an average of 500,000 yearly mastication cycles [29,30,31].

This study used an ex-vivo model and two assessment methods, colony-forming unit (CFU) counts and fluorescence microscopy, to assess the antimicrobial effects of bioceramic sealers modified with piezoelectric particles fillers on root dentin infected with *E. faecalis*. The antimicrobial activity of the sealers was assessed in direct contact with root dentin since dentin acts as a physical barrier and prevents the penetration and diffusion of antimicrobial agents [22,23]. Due to this limitation, we found it necessary to assess the antimicrobial activity of the modified sealers under the impact of dentin. In this study, *E. faecalis* was selected as a representative endodontic pathogen because it is commonly

found in chronic endodontic infections, and it is capable of surviving in the harsh and low-nutrient environment of the treated root canals [12]. Counting the colony-forming units (CFUs) is the gold standard for quantifying live bacteria. This method has two main advantages: it can count any quantity of bacteria and only counts viable bacteria by excluding dead bacteria and debris. However, a disadvantage of the CFU method is the possibility of miscounting clumps of bacterial cells as individual colonies. Additionally, obtaining results typically takes 1–3 days, which makes the technique unsuitable for serial longitudinal studies [32].

Although the bioceramic sealer alone has some antimicrobial effect against *E. faecalis*, this study demonstrated that incorporating BaTiO3 into the bioceramic sealer reduced the CFU count by nearly 2 log CFU compared to the control group. In contrast, the reduction in CFU for the bioceramic sealer without added particles was less than 1 log CFU compared to the control. Montoya et al. similarly observed a 2-log reduction in CFU against *S. mutans* using BaTiO3. Additionally, a study by Krishen et al. showed comparable antimicrobial effects against E. faecalis by incorporating Chitosan and Zinc Oxide particles into the endodontic sealer.

Fluorescence confocal microscopy provides significant advantages for examining viable bacteria, including high-resolution imaging and the ability to distinguish between live and dead cells in biofilms. In this study, the results of the CFU tests were verified using fluorescence confocal microscopy. Our findings revealed that all types of sealers significantly decreased the number of viable bacterial cells in dentinal samples compared to the control group. Furthermore, adding piezoelectric barium titanate nanoparticles ($BaTiO_3$) to the bioceramic sealer enhanced its antibacterial effect, with higher concentrations of

$BaTiO_3$ leading to a greater reduction in viable bacterial cells. The findings from fluorescence microscopy supported the CFU results, demonstrating more nonviable cells in bioceramic and modified sealers compared to the control group.

This study provides valuable insights into the antimicrobial activity of bioceramic sealers modified with $BaTiO_3$ on *E. faecalis*-infected root canals. Using CFU counts and fluorescence microscopy, we provided a comprehensive analysis of viable and nonviable biofilms, demonstrating the robust antimicrobial effects of the modified sealers. The findings emphasize the effectiveness of the bioceramic sealers in reducing bacterial viability and demonstrate the enhanced antimicrobial effects achieved by incorporating BTO nanoparticles. The findings suggest that increasing the concentration of BTO can improve the antimicrobial efficacy. The results of this study suggest a promising approach for creating high-performance endodontic sealers that can reduce bacterial cells in root canal-treated teeth for clinicians seeking more effective strategies to combat persistent endodontic infections.

CHAPTER 7

LIMITATIONS AND FUTURE WORK

Despite the significant findings of this work, this research had various limitations. The first limitation is that it could not normalize the amount of debris extracted from each canal by weight to control the precise amount of debris collected from each canal. Although a #4 round bur was used to full depth along the canal in all canals, the collected debris was not strictly equal in all canals. The lack of consistency in the amount of debris might have affected the number of bacterial colonies and potentially influenced the overall results. Furthermore, the mechanical forces that mimic mastication were not applied to all the samples in our study. Applying these forces to all specimens and assessing their potential impact on bacterial growth would be beneficial. Additionally, we only used 200 nm $BaTiO_3$ particles. Future studies should evaluate the impact of different particle sizes on the antibacterial effect and the flow properties of the bioceramic sealer. Lastly, studying the impact of the $BaTiO_3$-incorporated sealer and electrical charges on periapical tissues, bone regeneration, and mineralization would be beneficial. Understanding the effect of the sealer on periapical tissues could provide valuable insights into the biocompatibility and therapeutic potential of the sealer and could reveal any possible inflammatory responses in the periapical tissue. Investigating its role in bone regeneration and mineralization would help determine if the sealer can promote bone healing and enhance the overall success of endodontic treatments. This comprehensive evaluation could lead to improved clinical outcomes and broaden the application of $BaTiO_3$-incorporated sealers in dental practice.

CHAPTER 8

CONCLUSION

In this study, we incorporated piezoelectric particles of $BaTiO_3$ into Endosequence bioceramic sealer in two different concentrations (5%wt and 10%wt) and investigated their ability to reduce *E. faecalis* biofilm growth using an ex-vivo model. All the resulting sealers showed an antibacterial effect. Compared to the other groups, the best antimicrobial effect was evident in BC+10%wt $BaTiO_3$. Flow tests were also conducted. Results showed that sealers with 5%wt and 10 % wt $BaTiO_3$ both remained within the acceptable ISO limit for flow (diameter>17mm). However, adding more than 10% of $BaTiO_3$ may negatively affects the flow properties of the sealer.

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