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Shear Bond Strength Comparison of Bioceramics to Root Dentin.

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Shear Bond Strength Comparison of Bioceramics to Root Dentin.

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Thesis submitted to the School of Dentistry at West Virginia University in partial fulfillment of the requirements for the degree of

Master of Science in Endodontics

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Abstract

Shear Bond Strength Comparison of Bioceramics to Root Dentin

Yasmin Hoffman, D.M.D.

Introduction: The purpose of this study is to compare the push-out bond strength of four bioceramic materials to root dentin that are used during regenerative endodontics. The bioceramics being tested are: 1. Biodentine®(Septodont); 2. EndoSequence BC Putty Fast Set® (Brasseler); 3. NeoPutty® (Avalon Biomed); and 4. ProRoot MTA White® (Dentsply). A high bond strength of a bioceramic material to root dentin will provide excellent seal. An improved seal may correlate with less potential for contamination of the pulp space that is undergoing regenerative activity. This in turn will lead to long-term clinical success. The null hypothesis is that there is no difference in the mean bond strength of the four Bioceramic materials to dentin. The alternative hypothesis is that there is a significant difference among the mean push-out bond strength among the four materials investigated.

Methods: One hundred 2.0 mm thick dentin slices from human teeth were created using a 0.3 mm thick diamond cut-off wheel. For standardization, the canal space of each slice was prepared to a diameter of 1.5 mm. The cavity preparations were conditioned with 1.5% NaOCl, 17% EDTA, and saline. The slices were randomly separated into four groups: Biodentine® (n = 20); EndoSequence BC Putty Fast Set® (n=20); NeoMTA Plus® (n=20); and ProRoot MTA White® (n=20). Portland cement was used as the positive control (n=10). Cavit®, with no significant bond strength to dentin was used as the negative control (n=10). The push-out bond strength values were measured using a universal testing machine. A load was delivered to the cement surface by applying downward pressure with a 1.2 mm diameter cylindrical stainless-steel plunger at a crosshead speed of 0.5 mm/min. The nature of bond failure was inspected under a surgical microscope at 6.4x magnification. Failure pattern were categorized as adhesive failure, cohesive failure, or mixed failure. R software was used for all statistical analyses. The data were analyzed using a robust linear mixed-effects model to compare the mean fracture resistance of the samples. Statistical significance was defined as a $p < 0.05$.

Results: The mean push-out bond strength \pm standard deviation in MPa values of EndoSequence BC Putty Fast Set®; ProRoot MTA®; Biodentine®; NeoPutty®; Cavit®; and Portland Cement were 16.03 ± 4.05 , 10.69 ± 3.00 , 8.63 ± 3.73 , 7.29 ± 2.27 , 1.07 ± 0.53 , and 10.71 ± 3.47 respectively. The push-out bond strength of the EndoSequence BC Putty Fast Set® was significantly greater than the other experimental and control groups ($p < 0.05$). Inspection of the samples revealed the bond failure of the bioceramics to be predominantly cohesive failure.

Conclusion: Bond strength of a material plays an important role in clinical practice. The proper adhesion of bioceramics with dentin is critical. It is crucial to understand how bioceramics adapt and bond to dentin as the majority of endodontic failures are related to bacteria and their toxins entering the periapical tissues due to microleakage. The findings of the present study imply that the push-out bond strength of EndoSequence BC Putty Fast Set® was statistically greater when compared to Biodentine®, NeoPutty®, ProRoot MTA®, Cavit®, and Portland Cement. The majority of bioceramic showed cohesive bond failure, while the positive and negative control groups exhibited majority adhesive bond failure.

Dedication

I dedicate this thesis to God Almighty, who is my creator, my staunch supporter, and my fountain of inspiration, wisdom, knowledge, and understanding. Throughout this program, He has been my pillar of strength, and on His wings only have I soared. This work is also dedicated to my mother Alphonsine, who instilled in me the importance of perseverance and whose love for me had no boundaries. To my Husband, Noah, your constant support and encouragement during the doctoral program goes beyond what words can adequately express. You have given me the drive and discipline to tackle any task with enthusiasm and determination. I am truly thankful for having you in my life. To my lovely children, whom I can't force myself to stop loving. Everything I do in this life is for you. My love for you can never be quantified. You have made me stronger, better and more fulfilled than I could have ever imagined. God bless you.

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Graph 1. Push-out bond strength.....

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List of Symbols, Abbreviations or Nomenclature

Mineral Trioxide Aggregate - MTA

EndoSequence Bioceramic Putty Fast Set®- ERRM

Biodentine® – BIOD

NeoPutty® – NEOP

Portland Cement - PC

Sodium hypochlorite - NaOCl

Ethylenediaminetetraacetic acid – EDTA

White MTA® - WMTA

Bone Morphogenetic Protein 2 - BMP-2

Calcium Hydroxide – CH

Calcium Silicate Cement – CSC

Calcium Silicate Hydrate - CSH

Transforming Growth Factor Beta-1 - TGFβ-1

Interleukin 1 and 2 – IL-1 and IL-2

Macrophage Colony-Stimulating Factor - MCSF

Regenerative Endodontic Therapy - RET

Millipascal - MPa

Millimeters - mm

Microns – nm or μm

Newton - N

Chapter I

INTRODUCTION

An integral component of endodontic therapy is to eliminate and prevent microorganisms and their toxins from entering the root canal system and into the periapical tissues.¹ A sound coronal seal is crucial to endodontic success.² An endodontic failure may occur over time if the coronal portion of the tooth is not sealed with a material that bonds to the tooth and is resistant to dissolution by oral fluids. As a result of an improved seal, the pulp space that is undergoing endodontic therapy may be less susceptible to contamination.³ Quality seals improve the possibility of long-term clinical success.

Endodontic treatment depends on various bioceramic materials to establish a satisfactory coronal seal. According to Quershi, an ideal pulp capping material stimulates reparative dentin development, maintains pulp vitality, is bactericidal, adheres to dentin/restorative materials, is sterile, radiopaque, creates a bacterial tight seal, and withstands occlusal forces.⁴ There are three major classifications of bioceramics: bioinert, bioactive, and biodegradable. Bioceramics are biocompatible, non-toxic, radiopaque, provide hermetic seals, are antibacterial, can chemically bond with dentin, and are dimensionally stability. Similar to hydroxyapatite, these materials are osteoconductive and osteogenic and may induce regeneration processes in the human body.⁵ Bioceramics have several applications in endodontics. They can be used to repair perforations, act as root-end filling material, pulpotomies, endodontic sealers, and regenerative aids.⁶

Regenerative endodontics is a fascinating and rapidly developing field for the treatment of immature teeth with infected root canals and arrested root development and bioceramics are integral in the treatment. Guided stem cell engineering is used in regenerative endodontics to treat necrotic immature permanent teeth. This enables the root to continue to grow, apical bone

to heal, the dentinal wall to thicken, and apical roots to close. For stem cells to proliferate or mature *in vivo*, a conducive environment is necessary. To avoid pulp contamination, a well-sealed repair must be present *in vivo*.⁷ A review of case studies by Law revealed a correlation between successful regenerative endodontic treatments and effective coronal seal after treatment. The objective is to prevent microbial invasion of the pulp space by producing a "double seal" with a selected bioceramic and covering it with a bonded restoration. This allows for the complete revascularization of the pulp-dentin complex.⁸

Verma et al. reported in 2017 that residual bacteria had a substantial negative effect on the amount of root growth as determined by radiographs and the amount of dentin-related mineralized tissue produced.⁹ In addition, Conde et al. observed that reinfection of the root canal was responsible for the majority of root canal revascularization failures. The results of past studies are pertinent to the present analysis and highlight the necessity for a bacterial-proof barrier following regeneration procedures. After cleansing the canal and establishing a blood clot, it is necessary to place a barrier over the blood clot.¹⁰ Today, the recommended material for this application included bioceramics.

Mineral trioxide aggregate (MTA) was the first bioceramic introduced at Loma Linda University in the early 1990s. MTA is a powder comprised of hydrophilic nanoparticles that solidify in the presence of water. Tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium silicate, tetracalcium, aluminoferrite, and calcium sulfate dihydrate are the components of MTA.¹¹ MTA has been the material of choice for pulp capping in regenerative endodontics since its development.¹² In 2004, Trope and Banchs used MTA to revive immature permanent teeth with apical periodontitis. In spite of the fact that MTA is biocompatible,

bioinert, and bioactive, it has disadvantages, including a lengthy setting time, high cost, and the possibility of tooth discoloration.¹³

Researchers and chemists continued to improve MTA and developed newer bioceramics with improved qualities. Although MTA has had extensive research, the newer products lack the same level of evidence. This research evaluates four Bioceramic materials: Biodentine® (Septodont), EndoSequence BC Putty Fast Set® (Brasseler), NeoPutty®(Avalon Biomed), and ProRoot MTA® (Dentsply).

Biodentine® consists of two components. The powder component is composed of tricalcium silicates, dicalcium silicates, calcium carbonate, zirconium oxide, calcium oxide, and iron oxide. Calcium chloride is utilized in the liquid as a setting accelerator and water as a reduction agent.¹⁴

Endo Sequence BC Putty Fast Set® is a quick-setting putty composed of zirconium oxide, calcium silicates, tantalum oxide, monobasic calcium phosphate, and thickeners.¹⁵

Fine powdered tricalcium and dicalcium silicate, tantalite, calcium sulfate and silica make up NeoPutty®.¹⁶

Tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium silicate, tetracalcium, aluminoferrite, and calcium sulfate dihydrate are the components of ProRoot MTA®.¹¹

Bioceramic bond strength to dentin is important as they have significant adhesion which avoids microleakage.¹⁷ The bond strength of bioceramics to dentin may be measured utilizing a number of tests. These investigations include push-out, shear, and tensile strength testing, among others.¹⁸ The push-out test is a highly successful test to measure bond strength.

The push-out test involves application of shear force on the dentin's contact with the desired substance.¹⁹ The push-out test is often conducted in a manner comparable to clinical

applications in that the test materials are inserted into tubular cavities, similar to canal spaces. In push-out tests, the applied load is perpendicular to the dentinal tubules, replicating clinical pressures. The test allows for adequate specimen standardization, delivers more pure shear forces, and exposes the bonding inter-surface to less stress during sample preparation than conventional tensile and shear bond testing.²⁰ One benefit of the push-out test for evaluating bond strength is its ability to produce a uniform shear force and adhesive failure pattern on the samples.²¹

To date, there have been relatively few studies evaluating the shear bond strength of bioceramics to dentin. My review of the literature revealed that no study has examined Biodentine®, EndoSequence Fast Set BC Putty®, NeoPutty®, and ProRoot MTA®, in the same investigation. However, there have been studies in which a number of these products were compared to one another and various restorative materials. According to Majeed, Biodentine® and ProRoot MTA® demonstrated better bond strength and microhardness than BioAggregate®.²² Kaup observed Biodentine® exhibited equivalent shear bond values to glass ionomer cement after seven days, however MTA's® shear bond values remained considerably lower after fourteen days. The surface adherence of Biodentine® to dentin was superior than that of MTA.®²³ Similar findings were made by Pradeep, namely that Biodentine® had a stronger bond strength than ProRoot MTA®²⁴. Alsubait suggested that the force necessary to displace Biodentine® is comparable to White MTA® and much more than the force required to dislodge Bioaggregate®.²⁵ Ertas determined that the push-out bond strength of MTA varied by brand, with ProRoot MTA® exhibiting the greatest push-out bond strength.²⁶ According to Rahoma, Ortho MTA®, MTA Angelus®, and ProRoot MTA® materials exhibited comparable push-out bond strength values in root dentin.²⁷ Saghiri found that the force required to displace nano-

modified WMTA® was substantially more than that required for Angelus WMTA® and Bioaggregate®.²⁸

Adhesion between bioceramics and dentin is crucial. There are two major benefits of employing bioceramic materials: 1) The biocompatibility of the material prevents the surrounding tissues from rejecting them; 2) bioceramic materials include calcium phosphate, which improves their setting properties and results in a biochemical makeup and crystalline structure similar to that of tooth and hydroxyapatite, hence enhancing dentin bonding.²⁹ Since most endodontic failures are caused by bacteria and their toxins entering the periapical tissues as a result of microleakage, it is essential to understand how bioceramics adapt and attach to dentin. Hence, the purpose of this research is to assess and compare the shear bond strength of Biodentine®, EndoSequence BC Putty Fast Set®, NeoPutty®, and ProRoot MTA® to dentin.

Significance of the study

The aim of the study is to assess the bond strength of four distinct bioceramic materials to dentin. If one or more of the examined materials have a higher bond strength, this might indicate that a better coronal repair seal is feasible. A better seal may be associated with a reduced risk of pulp space contamination during endodontic therapy. Due to improved dentin bonding, endodontically treated teeth may be more resistant to forces induced by occlusion and attain a longer clinical life. The overarching goal is to use materials that reduce the amount of microleakage by enhancing the dentin-bioceramic material interface. This research will add to the literature bond strength information that could help clinicians make their choices for a bioceramic material to use during Regenerative Endodontics.

Statement of the problem

Microleakage is regarded as the single most critical risk factor for apical periodontitis. Endodontic treatment will be less successful if a material does not effectively adhere to dentin. A review of the literature has been conducted on the bond that occurs between bioceramics and dentin. There is a lack of information about bond strengths among four commonly used bioceramic materials.

Hypothesis

Bioceramic materials have varying dentin bond strengths. A Bioceramic material with a high bond strength to dentin will create an effective seal. An improved seal may be associated with a lower risk of contamination of the pulp space that is undergoing regenerative activity. This will, in turn, lead to long-term clinical success.

Null hypothesis

The null hypothesis is that there is no difference in push-out bond strength among Biodentine® (Septodont); EndoSequence BC Putty Fast Set® (Brasseler); NeoMTA Plus® (Avalon Biomed); ProRoot MTA White® (Dentsply).

Alternative Hypothesis

The alternative hypothesis is that there is a significant difference in mean push-out bond strength among the four bioceramics being tested.

Assumptions

1. The Instron machine accurately measures shear bond strength in a controlled laboratory environment.
2. The simulated cavity made by the operator is representative of a biologic space.

Limitations

1. There are intrinsic biologic variances, such as size, anatomy, calcification, between teeth from various dentitions.
2. The teeth were removed and preserved in saline for various amounts of time.
3. The *in-vitro* environment is not the true replication of the oral cavity.
4. There are minor differences across samples, due to human error during fabrication.
5. Sliding friction, rather than real bond strength, contributes significantly to dislocation resistance.
6. The elastic modulus of various materials may vary.

Delimitations

1. Only mandibular central and lateral incisors that have a round cross sectional canal space were used. Teeth used were devoid of fractures, carious lesions, or restorations.
2. The teeth were measured with a digital caliper, and any teeth that differed by more than 25% from the mean dimension value in length, mesiodistal width, and buccolingual width were eliminated.
3. To guarantee uniformity, sample preparation was performed by the same provider. All materials were mixed and applied to the cavity following manufacturer's instructions.

4. During experiment, all teeth specimens were kept in saline.
5. The Instron probe was placed in the center of all specimens to provide consistent loading.
6. In order to limit the contribution of frictional sliding to dislocation resistance, the shape of the root canal in the dentin slice should diverge in the forward direction of the applied load.

Chapter II

LITERATURE REVIEW:

- 1. Bioceramics Overview**
 - a. ProRoot MTA White® (Dentsply)**
 - b. Biodentine® (Septodont)**
 - c. EndoSequence BC Putty Fast Set® (Brasseler)**
 - d. NeoPutty® (Avalon Biomed)**
 - e. Portland Cement**
 - f. Cavit®**
- 2. Bioceramics in Regenerative Endodontics**
- 3. Push-out test**

Bioceramics are comprised of inorganic materials such as alumina and zirconia, bioactive glass, coatings and composites, hydroxyapatite and resorbable calcium phosphates.²⁹ According to Cheng et al., the extraordinary biocompatibility of bioceramics is a result of their resemblance to the biological process of hydroxyapatite production and their capacity to promote a healing response. They have osteoinductive ability because during the bone healing process, they absorb osteoinductive compounds. According to one proposed mechanism, osteoinductive biomolecules such as bone morphogenetic protein 2 (BMP-2) are absorbed into the surface of calcium phosphate bioceramics upon implantation, resulting in the initiation of bone formation and osteoinduction.³⁰

Bioceramics are also considered antibacterial due to the occurrence of *in-situ* precipitation during the material's setting phase, which results in the sequestration of bacteria. Bioceramics are porous powders that include nanocrystals ranging in size from 1 to 3 nm that hinder bacterial adhesion. Occasionally, apatite crystals include fluoride ions, and the resultant nanomaterial has antibacterial properties.³¹ Although the specific technique by which bioceramics attach to root dentin is unknown, the following hypotheses have been proposed to

explain the phenomenon. Sealer particles diffuse into dentinal tubules through tubular diffusion, resulting in mechanical interlocking connections. After denaturing collagen fibers with a strong alkaline sealer, there is mineral penetration into intratubular dentin, resulting in the creation of a mineral infiltration zone. The growth of hydroxyapatite along the mineral infiltration zone is a consequence of the partial reaction of phosphate with calcium silicate hydrogel and calcium hydroxide. This is produced by the reaction of calcium silicates in the presence of moisture from the dentin.³²

Mineral trioxide aggregate (MTA) is a biocompatible, hydrophilic endodontic material that promotes bone growth and repair. MTA is mainly composed of tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium silicate, tetracalcium, aluminoferrite, and calcium sulfate dihydrate.³³ When combined with water, calcium hydroxide releases calcium ions needed for cell adhesion and proliferation. In addition, the continual production of calcium hydroxide enables MTA to maintain a pH below 11.9, therefore fostering an antimicrobial environment.³⁴ MTA sets through an exothermic process that requires the hydration of its powder to produce a cement paste that hardens over time. The most major reactions include tricalcium silicate and dicalcium silicate reacting with water to create calcium silicate hydrates and calcium hydroxide. MTA's bioactivity is a result of the powder's hydration, which induces Ca^{2+} dissolution and diffusion. The formation of calcium silicate hydrates and calcium hydroxide leads to the production of hydroxyapatite. At 24 hours, MTA has a compressive strength of 40 MPa, and at 21 days, it has a compressive strength of 67 MPa.³⁵ Aggarwal V et al. discovered that MTA's push-out bond strength after 24 hours was 5.2 ± 0.4 MPa. After allowing the samples to set for 7 days, the strength increased dramatically to 9.0 ± 0.9 MPa.³⁶ MTA microhardness may be harmed by low humidity, low pH levels, the presence of a chelating agent, and increased condensation

pressure.³⁷ According to the majority of studies on dye and fluid filtration, MTA materials permit less total microleakage than conventional materials. MTA is not mutagenic, not neurotoxic, and has no detrimental effects on microcirculation.³⁷ In both animal and human research, the positive effects of MTA on the production of signaling molecules have been proven. The formation of calcium hydroxide, which releases calcium ions for cell attachment and proliferation, creates an antibacterial environment due to its alkaline pH, modulates cytokine production, promotes differentiation and migration of hard tissue producing cells, and forms hydroxyapatite on the MTA surface, which acts as a biologic seal.³⁸ MTA is one of the most often used calcium silicate-based bioceramics in endodontics, but it has a number of drawbacks, including a prolonged initial setting time of 78 minutes and final setting time of 261 minutes, poor handling characteristics, high cost, the absence of a known solvent, removal problems, and the risk of tooth discoloration.³⁹

Biodentine® was introduced to the market in 2009 as a fast-setting, bioactive alternative to dentin. Biodentine® is principally composed of highly refined tricalcium silicate (80.1%), calcium carbonate (14.9%), and zirconium oxide as a radio-opacifier. The liquid consists of calcium chloride as a setting accelerator, and a water-reducing component in the form of a hydro-soluble polymer.⁴⁰ Biodentine® has a maximum working time of 6 minutes, an initial setting time of 9–12 minutes, and a final setting time of 45 minutes. This is due to the addition of calcium chloride to the mixing liquid. A process of hydration hardens Biodentine®.⁴¹ When the powder reacts with the liquid, it produces silicate hydrate gel and calcium hydroxide (CH) as a byproduct. The pH and Ca^{2+} concentrations rise when CH dissociates into hydroxyl (OH^-) and calcium ions (Ca^{2+}).⁴² Calcium silicate cements emit Ca^{2+} , which enhances their bioactivity and apatite-forming abilities. Ca^{2+} activates the differentiation capacity of dental pulp cells and

promotes mineralization, which leads to the long-term creation of a dentin bridge on the pulp surface.⁴³ Increased Ca^{2+} release can also indicate OH^- release. OH^- has been shown to raise the pH of the surrounding tissue while also enhancing Biodentine's® antibacterial properties. Tissue healing has been shown to be triggered and aided by an alkaline environment.⁴⁴ Biodentine® may adhere to dentin because of the physical process of crystal formation inside dentinal tubules.⁴⁵ Han and Okiji found the development of tag-like structures extending from the material into dentinal tubules.⁴⁶ After one month, the compressive strength of Biodentine® is 300 MPa. This value is stable and falls within the range of the compressive strength of natural dentin, which is 297 MPa.⁴⁷ According to Ranjan, the elastic modulus of Biodentine® is 22.0 MPa, which is very similar to that of dentin, 18.5MPa. In addition, he observed that Biodentine® had a greater push-out bond strength than MTA, and that blood contamination had no effect on push-out bond strength regardless of setting time.⁴⁸ Biodentine® is non-toxic and has no negative effects on the differentiation or function of cells. Increasing pulp cell Transforming Growth Factor β -1 (TGF β -1) secretion stimulates angiogenesis, progenitor cell recruitment, cell differentiation, and mineralization. Biodentine® is simpler to manipulate than MTA, has superior mechanical properties, does not require two-visit appointment, and sets in 12 minutes, hence lowering the risk of bacterial contamination.⁴⁹

EndoSequence BC Putty Fast Set® (ERRM) is relatively new to the marketplace and is advertised as a pre-mixed bioceramic delivered in a moldable putty form. It's chemical composition consist of calcium silicates, zirconium oxide, tantalum oxide, calcium phosphate monobasic, and thickening agents.⁵⁰ ERRM is hydrophilic, insoluble, radiopaque, and aluminum free with a high pH, and require moisture to set and harden. It has been reported to have a twenty-minute setting time and a high cell adhesion capacity, allowing for quicker healing.⁵¹

According to Martinez-Cortés, ERRM was biocompatible when examined for cell survival, apoptosis, and mitochondrial dehydrogenase in human periodontal ligament fibroblasts. Cells treated with ERRM remained alive, maintained fibroblast-like morphology, and exhibited increased metabolic activity.⁵² Its 12.8 pH is partially responsible for its antibacterial properties, which diminish over a seven-day period, rendering it very biocompatible.⁵³ Lovato revealed that ERRM had antibacterial activity against 10 clinical strains of *E. faecalis* during its setting reaction. During the observation period, 53 studies comparing the antibacterial sealing capability of ERRM were resistant to bacterial microleakage.⁵⁴ In a three-dimensional culture, ERRM displayed more osteoblast differentiation than MTA in a single investigation.⁵⁵ ERRM was created to counteract some of the challenging handling aspects of MTA. MTA must be combined with a sterile liquid to get the required consistency, while ERRM components are ready-to-use out of the box. ERRM exhibits much less coronal tooth discoloration than the MTA line of materials.⁵⁶

NeoPutty®, according to the manufacturer, is a bioactive material comprised of finely powdered tricalcium and dicalcium silicate, tantalite, calcium sulfate, and silica, with a quick hardening time and no dentin discoloration. NeoPutty® is composed of a water-free organic liquid for improved handling capabilities.⁵⁷ NeoPutty® is designed to harden in the presence of moisture from surrounding tissues, including apical tissues, dentinal tubules, and pulp.⁵⁸ NeoPutty® releases calcium and hydroxide ions from its surface, promoting the formation of hydroxyapatite to aid in sealing and healing. Gandolfi suggested that NeoPutty's® prolonged release of calcium ions over a period of 28 days has a crucial role in promoting endodontic and periodontal tissue regeneration, hence enhancing bioactivity and biocompatibility.⁵⁹ When applied, NeoPutty® is instantly wash-out resistant and dimensionally stable in order to offer gap-

free sealing. Jardine et al. found that NeoPutty® was ineffective against multispecies biofilm using an intraoral infection model of dentin biofilm.⁶⁰ Further experiment revealed that NeoPutty® did not disrupt the multilayer structure formed by an *E. faecalis* and *Candida albicans* dual-species biofilm. This is because the antibacterial activity of NeoPutty® could only be evaluated via a direct contact test on freshly mixed material. Consequently, it is likely that NeoPutty's® antibiofilm action will be diminished after setting. NeoPutty® is a novel calcium silicate-based cement that exhibits minimum water solubility (3% when set), dimensional stability, and negligible expansion upon setting. Additionally, it includes very fine tricalcium and dicalcium silicate particles.⁶²

Portland cement (PC), the most widely used type of cement, is a key component of concrete, stucco, plaster, mortar, and grout. Alite (tricalcium silicate, Ca_3SiO_5), belite (dicalcium silicate, Ca_2SiO_4), aluminite (tricalcium aluminate, $\text{Ca}_3\text{Al}_2\text{O}_6$), and ferrite (tetracalcium aluminoferrite, $\text{Ca}_2\text{AlFeO}_5$) are the major components of Portland cement.⁶³ The rationale behind PC's widespread applications, such as the repair of root perforation and resorption, pulpotomy, and vital pulp therapy, is its advantageous properties, which include antibacterial activity, biocompatibility, bio inductivity, non-cytotoxicity, good seal, acceptable setting time, and physical and mechanical characteristics.⁶⁴ As a hydraulic cement, Portland cement sets by combining the dry powder with distilled water. The majority of the in vitro investigations that tested Portland cement's resistance to bacterial infiltration, dye leakage, and scanning electron microscopy concluded that MTA and Portland cement had comparable sealing abilities. The modest expansion that occurs after setting of Portland cement may be a factor in its capacity to seal.⁶⁵ Portland cement's compressive strength gets stronger with time. Seven days after mixing, there was no difference between Portland cement and MTA in terms of strength

values.⁶⁶ Portland cement has physical, chemical, and biological properties similar to MTA. Portland cement's clinical use is restricted due to its heavy metal leaching, arsenic levels, tooth discoloration, and some mechanical property concerns; however, histological analysis has revealed positive results in terms of biocompatibility, differentiation, and proliferation of Human Dental Pulp Cells (hDPCs) with a negligible inflammatory reaction of the pulpal tissue.⁶⁷ To qualify as a dental material, endodontic medical grade cement must be made under tightly regulated conditions. There aren't many clinical trials for this cement despite substantial experimental and animal research. Therefore, it is not advised to utilize Portland Cement in a therapeutic dental environment.

For many years, Cavit® has been used as a temporary dental filling substance. Cavit® includes zinc oxide, calcium sulfate, zinc sulfate, glycol acetate, polyvinyl acetate, and polyvinyl chloride-acetate. Due to water absorption, Cavit® has a significant linear expansion. The cavity may become sealed as a result of this growth, which would increase Cavit's® ability to stop microleakage. Saliva has a role in the setting reaction, which is brought about by the interaction of water with zinc oxide and zinc sulfate as well as calcium sulfate. After being set, the values for compressive strength of 250 psi were obtained. Despite being rather low, these values are within the clinically acceptable ranges needed for a base to resist displacement during amalgam condensation. Cavit® was found to have an insufficient film thickness and significant hygroscopic expansion for use as a temporary crown cement.⁶⁸ Because of its subpar qualities, it served as the negative control.

Regenerative endodontics is an exciting and rapidly developing field in the treatment of immature teeth with infected root canals and arrested root development. Regenerative Endodontic Therapy (RETs) have been regarded as a paradigm shift in the care of these teeth and may result in additional root growth with apical closure, dentinal wall strengthening by deposition of hard tissue, and post-treatment vitality response.⁶⁹ Tissue engineering, or regeneration of tissues requires a tetrad of elements. Stem cells need a favorable environment to multiply or differentiate *in vivo*. The need of a well-sealed restoration *in vivo* to minimize pulp contamination cannot be overstated.⁷⁰ Creating a healing environment for regeneration so that sealing material may be put on top of the blood clot is one of the most essential goals of RET. The objective is to prevent microbial invasion of the pulp space by establishing a "double seal" using a bioceramic and a bonded restoration. This permits the pulp-dentin complex to undergo revascularization unhindered. The sealing material must prevent the passage of bacteria or toxins, be biocompatible, and promote cell proliferation and differentiation.⁷¹

A current understanding of these novel bioactive materials is required to ensure the selection of the most appropriate material in various clinical settings. Due to their potential to promote root development, bioceramics are suggested for endodontic regeneration. These materials are good for blood clot top-sealing because they have the ability to seal, induce cell proliferation, differentiation, and biomineralization. Bioceramics enhances cell adhesion, proliferation, and migration of mesenchymal stem cells.⁷² Calcium ions generated by Bioceramics during setting stimulate a number of signaling molecules that influence cell development and differentiation, including interleukins IL-1 and IL-2, transforming growth factor (TGF β -1), and macrophage colony-stimulating factor (MCSF).⁷³ Along with TGF β -1,

which is produced by pulp cells following direct contact of the pulp lesion with bioceramic material, the previously sequestered growth factor guides pulp stem cells to the site of damage. TGF β -1 induces the development of stem cells into odontoblast-like cells, culminating in the establishment of a dentin reparative barrier. Neovascularization must be accomplished prior to the development of a reparative dentinal bridge, which takes around six days, in order to assure proper pulp regeneration.⁷⁴ Bioceramic materials enhance the proliferation and differentiation of dental pulp stem cells, as well as reparative dentinogenesis.⁷⁵

Bond strength refers to the process of union between two surfaces with different molecular compositions as a consequence of chemical, physical, or mechanical stresses. The chemical composition of the bioceramic material and the surface of the dentin influence the adhesive strength of the substance.⁷⁶ The strength of a bond may be determined by dividing the initial mechanical force required to fracture the bond by its geometrically specified cross-sectional area.⁷⁷ The source and condition of the substrate, dentin depth, enamel prism and dentinal tubule orientation, pulpal pressure, storage media and time, specimen size and shape, thermal cycling and mechanical loading, elastic modulus of bioceramic, operator skill, loading device configuration, cross head speed, and gripping are a few of the variables that influence bond strength measurement.

The results of the bond strength test might be affected by the mechanical properties of the bioceramics. Typically, finite element analysis is utilized to determine how the modulus of elasticity of bioceramics impacts the stress distribution at the bonded contact. Consequently, the use of stiffer bioceramics may significantly increase bond strength values.⁷⁸ A material

characteristic of the connection between bioceramics and tooth structure that cannot be evaluated by strength-based testing. The size and form of the specimen, the material characteristics of each component of the bonded assembly, the method of load application, and the presence of defects within or across materials all influence the measured bond strength and the resulting failure mode.⁷⁹ The push-out test was used for this investigation because it is a commonly used technique for measuring the interfacial bond strengths of root canal filling materials to radicular dentin. Its primary advantage over previous bond testing techniques is its capacity to test a material inside a canal surrounded by dentin, mimicking the clinical use of the material.⁸⁰

Chapter III

MATERIALS AND METHODS:

The West Virginia University Institutional Review Board authorized this study, Protocol #2201495975. The Tooth Repository at West Virginia University was used to collect one hundred extracted permanent human mandibular central and lateral incisors.

Exclusion/Inclusion criteria

Teeth were extracted for reasons unrelated to this study, such as periodontal disease or orthodontics. Teeth were cleansed and kept in a 1% thymol solution to remove any residual tissue or plaque. Under an operating microscope (Global Surgical, St. Louis, MO, USA), the samples were evaluated for abnormalities and radiographed buccolingually and mesiodistally to confirm that only samples free of carious lesions, cracks, or restorations were included. Teeth with curved roots, open apices, or prior root canal procedures were excluded. The buccolingual and mesiodistal widths of the teeth were measured using a digital caliper at the cemento-enamel junction. The tooth length was determined by measuring it from the incisal edge to the apex. To guarantee the use of similar specimens, teeth that differed by more than 25% from the mean dimension value were excluded.

Sample Preparation

The selected one hundred teeth were prepared to obtain 2.0 mm thick dentin sections with the removal of the crowns. The middle thirds of the roots were sectioned horizontally using a 0.3 mm thick diamond cut-off wheel and slow speed rotary machine with water cooling system. This would allow us to obtain one sample per tooth. With 320-grit sandpaper, each cut surface was

polished. Each section's thickness was measured using a digital caliper to an accuracy of 0.01 mm. For standardization, the canal space of each slice was instrumented to achieve a diameter of 1.5 mm. To eliminate any debris, all samples were washed with saline. All samples were mounted in epoxy resin blocks. The cavity preparations were conditioned with 1.5% NaOCl, 17% EDTA and saline. The liquid existing in the canal walls were absorbed using paper points. The slices were randomly separated into four test groups (n = 20 for each group). Portland cement was used as a positive control (n=10) and Cavit, known to have poor bonding to dentin, was used as the negative control (n=10).

Group A: (20) Biodentine® (Septodont) + Dentin Slice

Group B: (20) EndoSequence BC Putty Fast Set® (Brasseler) + Dentin Slice

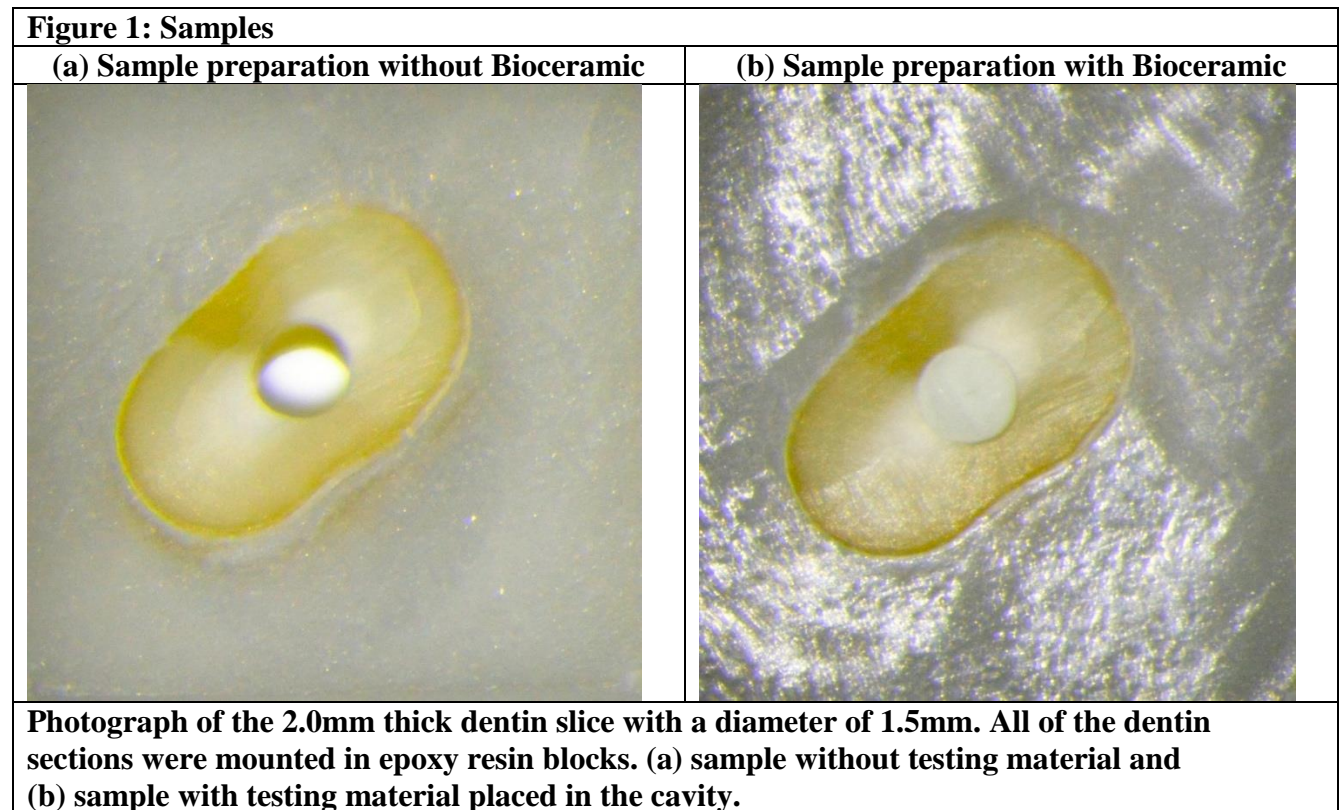
Group C: (20) NeoPutty® (Avalon Biomed) + Dentin Slice

Group D: (20) ProRoot MTA White® (Dentsply) + Dentin Slice

Group E: (10) Positive control (PC): Portland Cement + Dentin Slice

Group F: (10) Negative control (NC): Cavit® + Dentin Slice

All filling materials were prepared according to the manufacturer's directions before being inserted within the lumen of the slices and condensed with an endodontic plugger. The surplus material was removed using a scalpel. Specimens were wrapped in moist gauze and put in an incubator for 72 hours at 37°C and 100% relative humidity. Each group were put in its own closed plastic container. To maintain a sufficiently moist atmosphere, saline soaked pieces of gauze were replaced daily.



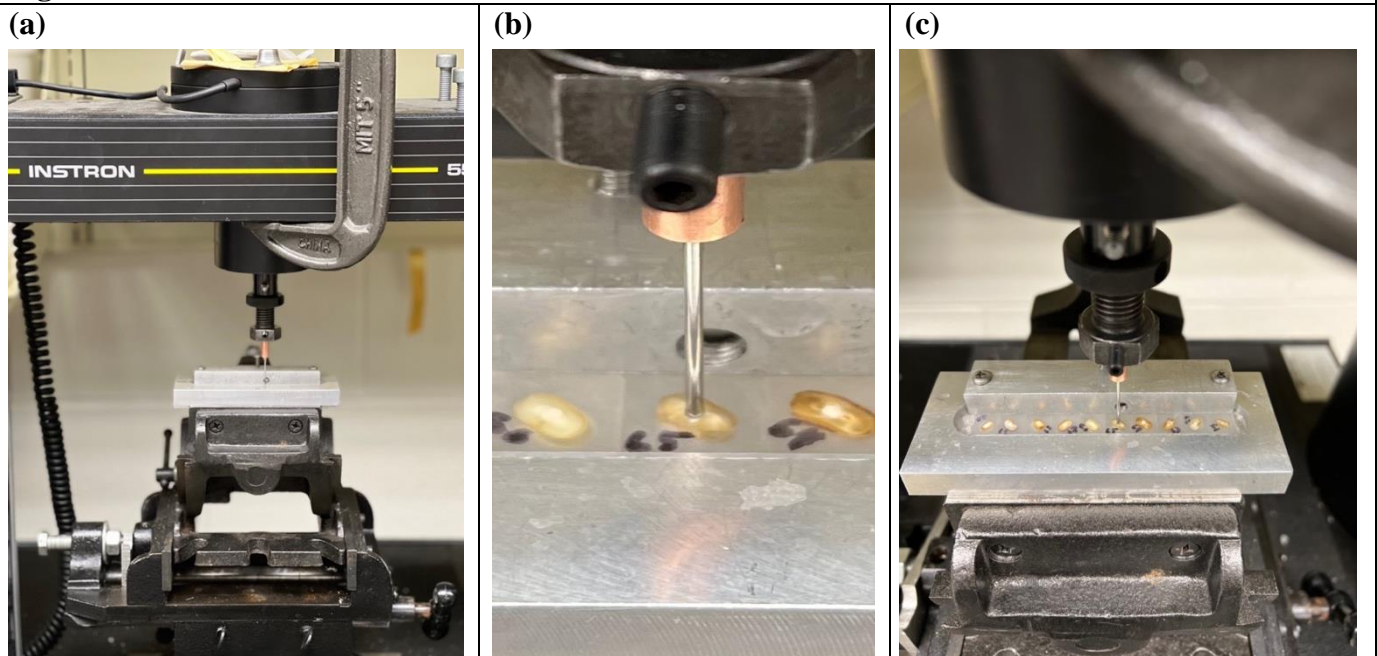
Push-out test

The push-out bond strength values were measured using a universal testing machine (Instron, Norwood, USA). The machine was calibrated before use. Each sample was carefully placed on a metal slab with a pre-drilled 1.5 mm center hole to allow the Instron's plunger to move freely. A load was delivered to the cement surface by applying downward pressure with a 1.2 mm diameter cylindrical stainless steel plunger at a crosshead speed of 0.5 mm/min. To ensure only contact with the bioceramic material, the plunger diameter was slightly less than the canal diameter. The maximal force used to dislodge the cement was measured in Newtons. The force was exerted until the bond failed completely, at which point it was measured in Newtons (N). The bond strength in MPa (millipascal) was calculated using the following formula:

$$\text{Bond Strength (MPa)} = \text{Debonding force (N)} / \text{Bonded surface area (mm}^2\text{)}$$

Bonded surface area = $2\pi rh$, $\pi = 3.14$ (constant), r = radius, h is the thickness of dentin section.

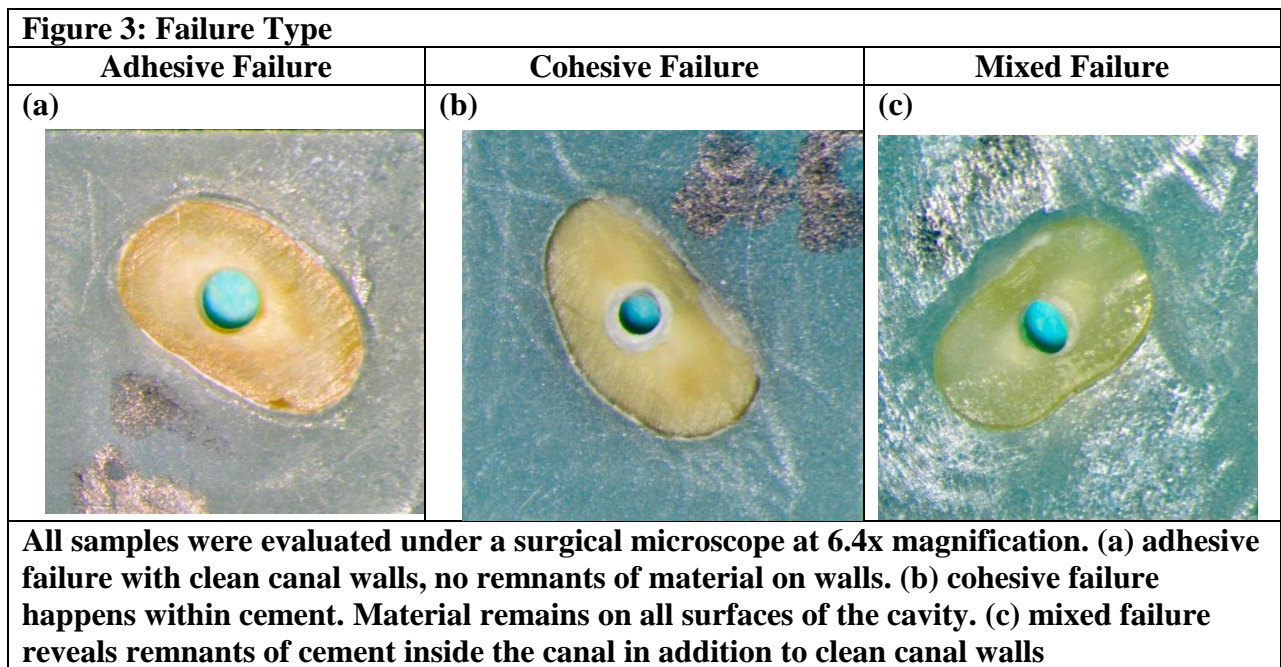
Figure 2: Instron Machine



Photograph of (a) the Instron machine with the samples being pushed out the test cavity (b) zoom in picture of custom-made stainless steel plunger aligned with the specimens in the center of the cavity (c) custom-made jig with ten specimens aligned before push out bond test.

Failure pattern

To determine the nature of bond failure, each broken sample was inspected under a surgical microscope at 6.4x magnification (Global Surgical, St. Louis, MO). Each sample was assigned to one of three failure categories: adhesive failure at the dentin-material interface, cohesive failure within the material, or mixed failure, a mixture of the two failure forms. The operator who analyzed the slices was not aware of which sample corresponded to which substance.



Statistical analysis

R 4.2.2 software (Vienna, Austria) was used for all statistical analyses. The data was analyzed using a robust linear mixed-effects model to compare the mean fracture resistance of the samples. Statistical significance was defined as $p < 0.05$.

Chapter IV

RESULTS:

PUSH-OUT TEST

The mean push-out bond strength and standard deviation of each tested material, highest strength to lowest strength are: 16.03MPa \pm 4.05 for EndoSequence BC Putty Fast Set (ERRM)[®], 10.69MPa \pm 3.00 for ProRoot MTA[®], 8.63MPa \pm 3.73 for Biodentine[®], and 7.29MPa \pm 2.27 for NeoPutty[®], The value for the controls were 1.07MPa \pm 0.53 for Cavit[®] (negative control), and 10.71MPa \pm 3.47 for Portland Cement (positive control). These values are presented in Table 1.

The mean push-out bond strength value of the EndoSequence BC Putty Fast Set[®] group was a higher than the other groups. $P < 0.05$ was considered statistically significant in the study. According to the analysis of variance, there was a statistically significant difference between the following groups Biodentine[®] and ERRM[®] ($p < .0001$), Biodentine[®] and Cavit[®] ($p < .0001$), ERRM and NeoPutty[®] ($p < .0001$), ERRM and ProRoot MTA[®] ($p < 0.0004$), ERRM and Cavit ($p < .0001$), ERRM[®] and Portland Cement ($p < 0.0034$), NeoPutty[®] and ProRoot MTA[®] ($p < 0.0031$), NeoPutty[®] and Cavit[®] ($p < .0001$), NeoPutty[®] and Portland Cement ($p < 0.0489$) and ProRoot MTA[®] and Cavit[®] ($p < .0001$). The weighted least square test revealed that the push-out bond strength of the EndoSequence BC Putty Fast Set[®] was significantly higher than the experimental and control groups ($p < 0.05$). Table 2 displays the significant analysis of variance between the experimental groups.

MATERIAL	#	MEAN	STANDARD DEVIATION	LOWER CONFIDENCE LIMIT	UPPER CONFIDENCE LIMIT
ENDOSEQUENCE BC PUTTY FAST SET®	20	16.03 MPa	4.05 MPa	14.28MPa	17.59MPa
PRO ROOT MTA®	20	10.69 MPa	3.00 MPa	8.96MPa	12.26MPa
BIODENTINE®	20	8.63 MPa	3.73 MPa	6.98MPa	10.29MPa
NEOPUTTY®	20	7.29 MPa	2.27 MPa	5.64MPa	8.95MPa
PORTLAND CEMENT	10	10.71 MPa	3.47 MPa	5.96MPa	16.77MPa
CAVIT®	10	1.07 MPa	0.53 MPa	.42MPa	1.92MPa

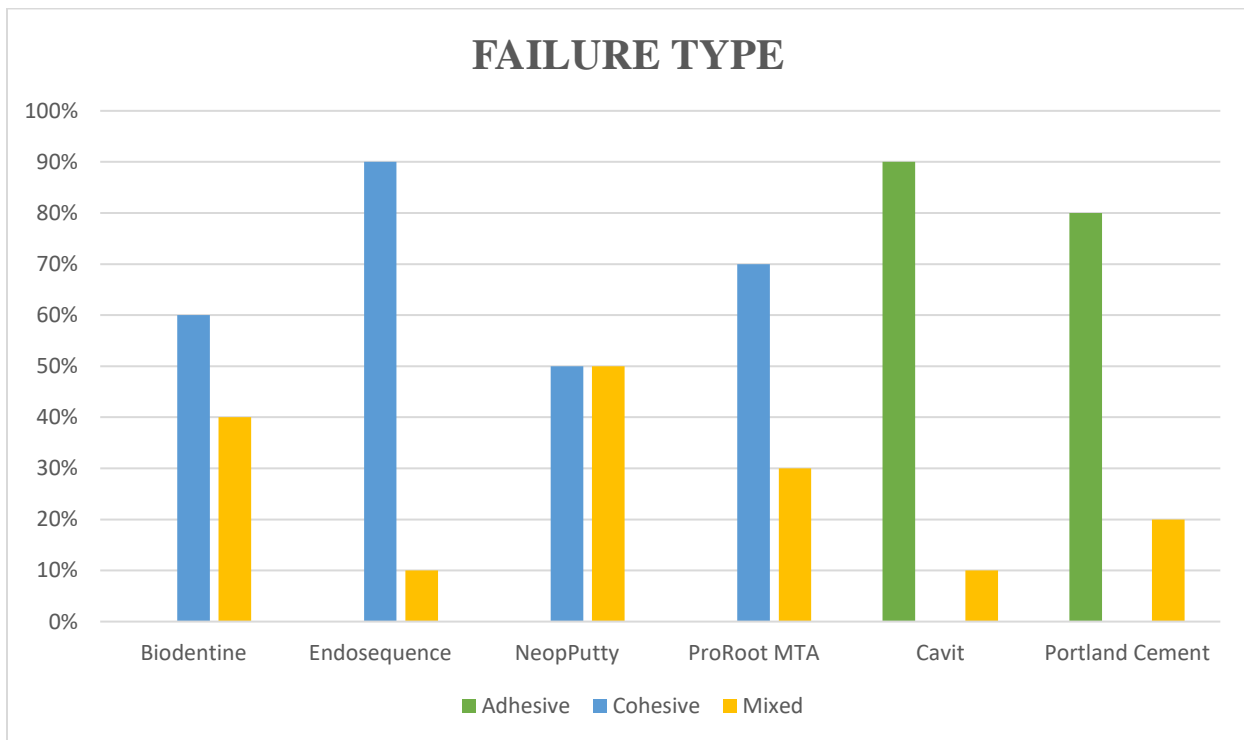
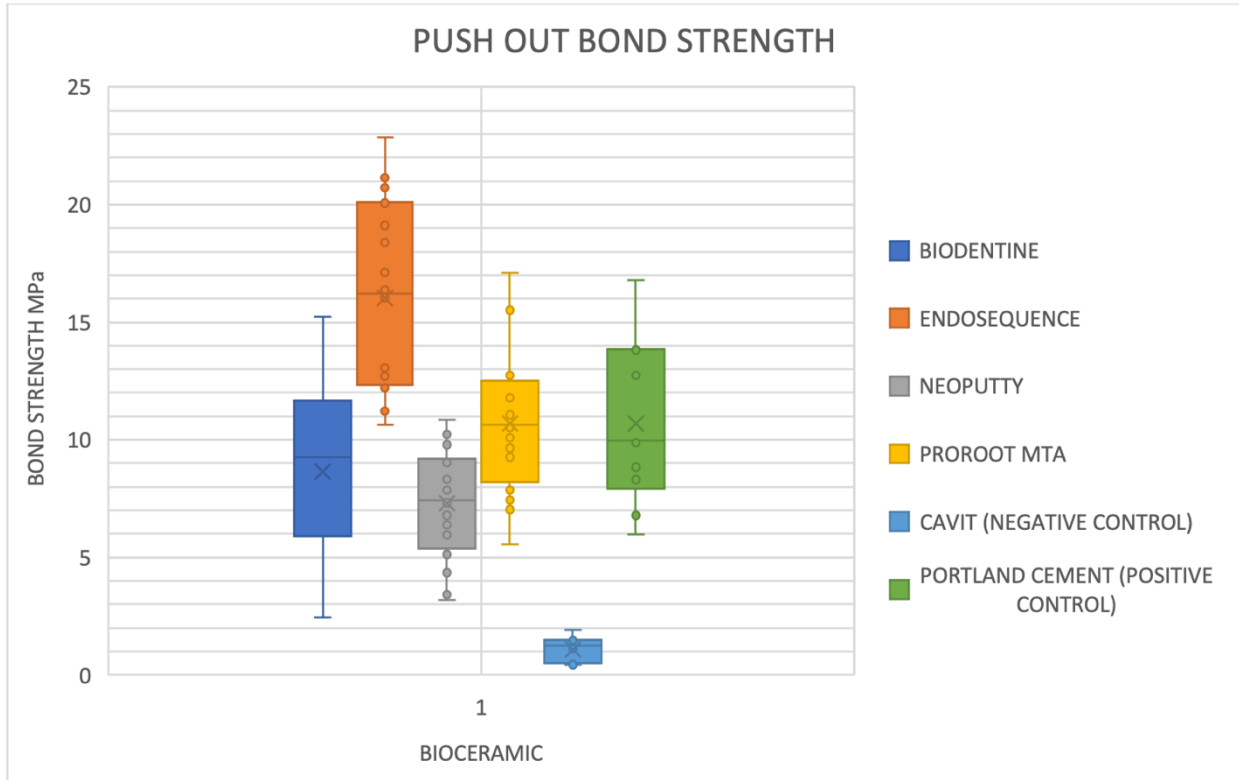
COMPARISON	DIFFERENCE, MPa	P-VALUE	Lower Confidence Limit	Upper Confidence Limit
Biodentine® as compared to EndoSequence®	-7.30	<.0001	-10.36	-4.23
Biodentine® as compared to NeoPutty®	1.34	0.6736	-1.72	4.40
Biodentine® as compared to ProRoot MTA®	-1.98	0.3457	-5.04	1.08
EndoSequence® as compared to NeoPutty®	8.64	<.0001	5.58	11.70
EndoSequence® as compared to ProRoot MTA®	5.32	<.0001	2.26	8.38
NeoPutty® as compared to ProRoot MTA®	-3.32	0.0275	-6.38	-0.25

*Note: The significant relationships are in bold.
Example in using the table: There is a significant difference in the bond strength between Biodentine® and EndoSequence® and the bond strength of Biodentine® are 7.3 units less than that from EndoSequence® (p<.0001, 95% CI: -10.36, -4.230)*

BOND FAILURE TYPE

The nature of failure within each specimen was evaluated under a surgical microscope at 6.4x magnification (Global Surgical, St. Louis, MO). The failure patterns are presented in Table 3 as adhesive failure, cohesive failure, or mixed failure. Adhesive failure is an interfacial bond failure between the adhesive and the adherend. Cohesive failure occurs when a fracture allows a layer of adhesive to remain on both surfaces. When the adherend fails before the adhesive, it is known as a cohesive failure of the substrate. Mixed failure occurs when there are remnants of the cement inside the canal. All samples were tested until they failed. Biodentine's® failure types were 60% cohesive failure and 40% mixed failure. EndoSequence BC Putty Fast Set® had 90% cohesive failure and 10% mixed failure. NeoPutty® had 50% cohesive failure and 50% mixed failure. ProRoot MTA® had 70% cohesive failure and 30% mixed failure. As expected for a negative control, Cavit had 90% adhesive failure and 10% mixed failure. Portland cement, the positive control, had 80% adhesive failure and 20% mixed failure. Overall, the majority of the Bioceramic materials had cohesive failure types.

Material	n	Adhesive failure,%	Cohesive failure, %	Mixed failure, %
Biodentine®	20	0%	60%	40%
EndoSequence BC Putty Fast Set®	20	0%	90%	10%
NeoPutty®	20	0%	50%	50%
ProRoot MTA®	20	0%	70%	30%
Cavit®	10	90%	0%	10%
Portland Cement	10	80%	0%	20%



Chapter V

DISCUSSION

An ideal bioceramic material used in Endodontics must possess the following properties: adhesion to dentin preventing leakage from the interface of the core material and dentin, decreased moisture sensitivity, insolubility, tissue inductive properties, resistance to occlusal forces, good physicochemical properties, and biocompatibility.⁸¹ The presence of persistent bacteria and their toxins in the peri radicular tissues, resulting in inflammation, causes the majority of endodontic failures. Bond strength is defined as the force required to break a bond divided by the bonding interface's cross-sectional area. The push-out test offers exceptionally precise data because failure occurs parallel to the material-dentin contact, which more closely resembles clinical settings.⁸²

The published literature on the bond strength of bioceramic putty is very limited. The values of push-out bond strength of bioceramics varies. Majeed et al evaluated the bond strength of Biodentine® and ProRoot MTA® and reported mean values of 42.02 MPa and 21.86 MPa respectively.⁸³ Whereas Pradeep found a bond strength value of Biodentine® and ProRoot MTA® to be 9.6 MPa and 7.7 MPa after 1 week respectively. Shokouhinejad reported a bond strength value of MTA and ERRM of 8.4 MPa and 17.79 MPa after one month respectively.⁵⁰ This study is the first to compare the push-out bond strength of Biodentine® (Septodont), EndoSequence BC Putty Fast Set® (Brasseler), NeoPutty® (Avalon Biomed), and ProRoot MTA® (Dentsply).

This study was completed in-vitro with coronal dentin slices that were fabricated alike. For standardization, all materials were tested after an 72 hours since mixing. This time frame allowed for adequate setting of all materials used in experiment. The push-out bond strength of

Biodentine®, EndoSequence BC Putty Fast Set®, and NeoPutty®, and ProRoot MTA® was examined in the present study. Mean bond strengths and standard deviations of the groups were: 8.63 ± 3.73 for Biodentine®, 16.03 ± 4.05 for EndoSequence BC Putty Fast Set®, 7.29 ± 2.27 for NeoPutty®, 10.69 ± 3.00 for ProRoot MTA®, 1.07 ± 0.53 for Cavit® (negative control), and 10.71 ± 3.47 for Portland Cement (positive control). ERRM had statistically significant higher push-out bond strength values than all other groups. The null hypotheses of the study was rejected because there were differences in push-out strength amongst samples. In addition, the alternative hypothesis was accepted since there were statistically significant differences amongst the experimental groups.

The material chemistry, the fine particle sizes, the low water-to-cement ratio, and the presence of calcium carbonate may have contributed to the ERRM group's superior bonding to dentin.⁸⁴ Calcium silicates, zirconium oxide, tantalum peroxide, monobasic calcium phosphate, and fillers comprise ERRM. During the setting of cement, tricalcium silicate hydrates to produce calcium silicate hydrate (C-S-H) gel and calcium hydroxide; moreover, calcium phosphate monobasic reacts with $\text{Ca}(\text{OH})_2$ to precipitate hydroxyapatite in situ inside C-S-H.⁸⁵ It is speculated that the thickening and filling chemicals used to give the calcium trisilicate putty its putty shape contributed to the improved bond strength of the putty form.⁸⁶ Materials with a quicker setting time release much less Ca^{2+} and have significantly lower Ca/P ratios. Due to the prolonged setting period of ERRM, more calcium ions are available and crystals precipitate more easily, resulting in a stronger bond.⁸⁷ By using zirconium oxide, some physical properties of bioceramics were improved. The composition and particle size of these bioceramics impact the interaction between cement and root canal dentin. Due to its minute particle size, ERRM may form chemical bonds with the dentin walls of the root canal, resulting in a strong bond.⁸⁸ Due to

ERRM's particle structure and hydrophilic qualities, its binding strength may be greater than that of the other materials evaluated.⁸⁹

Calcium silicate cement may adhere to dentin by a chemical bond or micromechanical anchoring through cement tags in the dentinal tubules, although the precise process is uncertain. It has been hypothesized that when calcium and hydroxyl ions are released from calcium silicate-containing material, they form a covering of hydroxyapatite when they come into contact with the fluids of the dentinal tubules. The presence of nanosphere particles with a diameter of $1 \times 10^{-3} \mu\text{m}$ allows for the material to enter dentinal tubules, interact with the dentin liquid and form a mechanical bond on complete set of the material. This interfacial layer forms a chemical bond between the calcium and dentin walls.⁹⁰ The capacity of a bioceramic to generate apatite is considered essential for the creation of a mineral-rich interfacial layer and a tag-like structure reaching from the interfacial layer to the dentinal tubule. This "mineral infiltration zone" has been documented in prior research, with the authors speculating that its occurrence is related to the development of hydroxyapatite at the interface between two substrates. The penetration of tubules and creation of tag-like structures increase the contact area between dentin and bioceramics, hence enhancing the seal and marginal adaptation.⁹¹

After inspecting the root sections, the study revealed that the two most prevalent failure modes among all bioceramics examined were cohesive and mixed. This conclusion is consistent with prior investigations on bioceramics that shown cohesive and mixed failures.⁹² Sixty percent of Biodentine's® failures were cohesive and 40% were mixed. The failure rate of EndoSequence BC Putty Fast Set® was 90% cohesive and 10% mixed. NeoPutty® had a failure rate of 50% cohesive and 50% mixed. ProRoot MTA® demonstrated a cohesive failure rate of 70% and a mixed failure rate of 30%. Cavit® exhibited 90% adhesive failure and 10% mixed failure. The

failure rate of Portland cement was 80% adhesive and 20% mixed. The adhesive failures indicate that material chemists may want to work to increase the property of chemically bonding between the root canal dentin and the bioceramic. The majority of recorded mixed failures may have been due to brittle cement or inadequate setting, resulting in poor physical properties. The varied particle sizes of the examined materials have an effect on how efficiently they enter the dentinal tubules and may be related to the observed differences in bond strengths and forms of bond failure.⁹³ The bond strength is deemed appropriate when the failure is cohesive inside the bioceramic material, indicating strong adhesion between the bioceramic material and dentin. Different intrinsic cohesive strengths of the bioceramic materials examined may account for the disparities in mode of failure. Ideally, you will need both material strength as well as bond strength.

The interfacial adaption of bioceramics is essential because they need to interact chemically with dentin to demonstrate sufficient bioactivity, whether they are utilized as a pulp capping agent, a root-end filling material, or a root canal sealer.⁹⁴ High bond strength to root dentin and compression resistance are recognized to be key characteristics for the effectiveness of regenerative endodontic operations. They prevent clinical failures of root canal recontamination by dislodging materials from stresses caused by occlusion or condensation of restorative materials.⁹⁵ The depth of penetration of the materials into the dentinal tubules and the length of the produced tags have not been evaluated. The variation in bonding strength levels could be explained by differences in the depth of penetration and microstructure of the tags created by the two materials.⁹⁶ However, further studies are required to identify the molecular interaction between the materials and dentin. The purpose of the study was to determine bond strength for regenerative procedures. Push-out testing is routinely used to determine the strength

needed to accommodate occlusal forces. It should be noted that in regenerative procedures, it is restorative materials and not sealants that are most responsible for response to occlusal forces. Nevertheless, having regenerative materials with strong adhesive qualities improves the potential for regeneration. This *in vitro* push-out study, although a simulation of the *in vivo* environment, is useful in identifying characteristics of common bioceramic materials available to clinicians.

Chapter VI

CONCLUSION

Under the parameters of this *in vitro* research, it can be stated that EndoSequence BC Putty Fast Set® had a significantly greater push-out bond strength than Biodentine®, NeoPutty®, and ProRoot MTA®. There was a statistically significant difference in push-out strength between NeoPutty® and ProRoot MTA®. There was no discernible difference between Biodentine® and NeoPutty® or Biodentine® and ProRoot MTA®. In the majority of the tested groups, cohesion failure patterns were seen. In this study, all examined bioceramics exhibited the production of crystalline structures resembling apatite that adhere to dentin. When a bioceramic is utilized for pulp capping during RETs, root end filling, or perforation seal, it must create an appropriate seal with excellent adhesion to dentin to prevent leakage. Consequently, the bond strength of the materials used plays an essential function in clinical practice. An ideal material needs to be a strong material to undergo occlusal pressure and restorative procedure. Strong adherence bond strength is only one aspect to improving the success of regenerative endodontics. Long-term clinical studies should be carried out to determine the success of the bioceramic materials.

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Conflicts of interest

There are no conflicts of interest.

Chapter VII

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