2023

An In Vitro Assessment of the Antibacterial Activity, Biocompatibility, and Mineralization Inducing Potential of Biodentine®XP

Chandani Arjun Mantri
University Of Alabama At Birmingham

AN IN *VITRO* ASSESSMENT OF THE ANTIBACTERIAL ACTIVITY, BIOCOMPATIBILITY, AND MINERALIZATION INDUCING POTENTIAL OF BIODENTINE® XP

by

CHANDANI ARJUN MANTRI

NATHANIEL C. LAWSON, COMMITTEE CHAIR
AMJAD JAVED
NATHAN SMITH
PING ZHANG

A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of Master of Science

BIRMINGHAM, ALABAMA

2023

AN IN *VITRO* ASSESSMENT OF THE ANTIBACTERIAL ACTIVITY, BIOCOMPATIBILITY, AND MINERALIZATION INDUCING POTENTIAL OF BIODENTINE® XP

CHANDANI ARJUN MANTRI

DENTISTRY

ABSTRACT

Purpose: The purpose of this study was to assess the *in-vitro* antibacterial activity, biocompatibility, and mineralization-inducing potential of Biodentine® XP. **Methods:** Four contemporary pulp capping materials, namely Dycal®, a calcium hydroxide, Theracal® LC, a resin calcium silicate and, tricalcium silicates namely Biodentine®, and Biodentine® XP were compared in the study. The antibacterial activity of the pulp capping materials included in the study was evaluated against *Streptococcus mutans* UA159 and *Enterococcus faecalis* ATCC 29212 and were determined by the direct culture test. The biocompatibility of these materials in preodontoblastic 17IIA11 cells were compared by the MTT assay and mineralization-inducing potential in preodontoblastic 17IIA11 cells were compared by quantifying calcium deposits on Alizarin Red Staining (ARS) on day 5. Data was expressed as mean ± SEM (standard error of mean) and was evaluated statistically by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism 9 (GraphPad Software Inc, San Diego, California). Differences between groups was considered significant at $p<0.05$. **Results:** In direct culture test, Biodentine® XP showed strongest antibacterial activity against *S. mutans* whereas Biodentine® and Biodentine® XP showed highest antibacterial activity against *E. faecalis*. However, all the tested pulp capping materials showed distinct antibacterial effects against *S. mutans* and

E. faecalis. Of the tested materials, acceptable biocompatibility was shown by Biodentine® XP and Biodentine®. Biodentine® XP, Biodentine® and Theracal® LC have shown favorable mineralization-inducing potential in our experiment. Of all the tested materials, Dycal showed the least antibacterial activity, biocompatibility, and mineralization-inducing potential. **Conclusion:** Biodentine® XP showed acceptable antibacterial activity, biocompatibility, and mineralization-inducing potential of all the tested materials**.** Therefore, the new material can be used as a pulp capping material owing to its wide array of clinical implications and ease of use. However, more research is necessary.

Keywords: Calcium hydroxide, Resin Calcium silicate, Calcium silicate, Pulp capping material, Antibacterial activity, Biocompatibility, Mineralization-inducing potential

DEDICATION

This dissertation is dedicated to:

My family and friends

For being my source of constant inspiration and strength.

Your unwavering support and endless love have been instrumental in my journey.

My mentors and professors

For sharing your knowledge and expertise with me and inspiring me to pursue a career in dentistry and research.

Your guidance and encouragement have been invaluable in shaping my professional growth.

This thesis is a testament to the hard work, dedication, and passion of all those who have supported me along the way.

ACKNOWLEDGMENTS

Firstly, I would like to thank Dr. Nathaniel C. Lawson, for accepting me into the program and the wonderful two years. I appreciate his mentorship, and guidance that helped me accomplishing the residency.

I would like to thank Dr. Ping Zhang for patiently mentoring the past one year and guiding me to accomplish the project. I appreciate her for training me in microbiology and cell culture, something that was new to me.

I want to thank my co-residents for the best two years in residency. I want to thank Tom Lawson and Greg Harbor for all the technical support in Biomaterials and Microbiology lab.

I would like to thank my committee members, Dr. Ping Zhang, Dr. Nathan Smith, and Dr. Amjad Javed for their advice and encouragement that helped bring this project together.

I would like to thank Ms. Phelecia Jemison, Ms. Brittany Conner, and Ms. Sheila Turner for all their help and advice with administrative work.

I would like to thank Dr. Lawson lab and Dr. Zhang lab for letting me use their lab space to complete the project. I would like to thank UAB School of Dentistry, the city of Birmingham and the state of Alabama for letting me call it ‘Home Sweet Home’ for the past two years.

And lastly, I would like to thank Septodont Inc. for sponsoring this project, supplying the materials for the research.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table *Page*

LIST OF FIGURES

Figure *Page*

LIST OF ABBREVIATIONS

AAE	American Academy of Endodontics
ADT	Agar Diffusion Test
ANOVA	Analysis of variance
ARS	Alizarin Red S Staining
Bis GMA	Bisphenol A-glycidyl Methacrylate
CFU	Colony Forming Unit
CH	Calcium hydroxide
CHC	Calcium hydroxide cements
CSC	Calcium silicate cements
CPC	Cetylpyridinium chloride
DCT	Direct Culture Test
E. faecalis	*Enterococcus faecalis*
HDPSC	Human dental pulp stem cells
MTA	Mineral trioxide aggregate

MTT	3-(4,5- dimethylthiazolyl-2)-2, 5-diphenyl-2H-tetrazolium bromide
OD	Optical density
PBS	Phosphate-Buffered Saline
RCSC	Resin calcium silicate-based cements
SEM	Standard Error of Mean
S. mutans	*Streptococcus mutans*
TGF-β1	Transforming factor beta 1

1. INTRODUCTION

Vital Pulp Therapy

The American Association of Endodontics (AAE) defines Vital Pulp therapy as, 'treatment aimed at preserving and maintaining pulp tissue that has been compromised by trauma, caries or restorative procedures in a healthy state' (1). The procedures commonly employed to maintain the pulp vitality are as follows: Direct pulp capping (1,3,8,16), indirect pulp capping, (1,2-3,16) and pulpotomy. (1,3-5) Haskell *et al.* (6) reported an 86% 12-year survival rate on pulp capping compared to root canal treatment.

Direct pulp capping is a treatment aimed to restore the tooth vitality with pinpoint pulpal involvement due to trauma, (1,7) carious or mechanical exposure, (1,8-9) A biocompatible material is placed on the exposed pulp which facilitates the reparative dentine formation. (7-10) However, increased risk of failure is observed in pulp tissue of the primary dentition due to higher cellular content. (9) Also, direct pulp capping is less commonly employed in primary dentition due to undifferentiated mesenchymal cells in the pulp of primary teeth that differentiates into odontoblastic cells. As a result, internal root resorption and acute dentoalveloar abscess can be observed. (10-11)

Indirect pulp capping is a treatment carried out in cases of deep caries, approximating the pulp, with no observed pulpal involvement radiographically. (3,8,12) It can either be a one-step or two-step procedure. In one-step procedure, the affected dentine that if removed will expose the pulp, is left behind and a pulp capping medicament is placed followed by placing a restoration on the same visit. (3,8,12-13) In a two-step procedure, the first appointment is similar to the one-step procedure except provisional restoration such as zinc oxide eugenol or glass ionomer cement is placed. In the second appointment, the caries should be arrested, and dentine should be remineralized. The tooth is reevaluated, and a final restoration is placed. (3,8)

Pulpotomy is a minimally invasive procedure in which the diseased or inflamed coronal portion of the pulp is removed, followed by placing a biocompatible material to maintain radicular pulp vitality and promote repair. (8-9,13). It can be of two types: complete pulpotomy and partial 'Cevk' pulpotomy. (8-9,13) In complete pulpotomy, the entire coronal portion of the pulp is removed, whereas, in partial pulpotomy 2–3 mm of the coronal pulp is removed. (8,13) In the past, formocresol has been widely used but several studies have found evidence of its association with mutagenic, toxic, and carcinogenic risks in humans. Due to potential risks associated with formocresol use, the use of biocompatible materials in pulpotomies has gained popularity. (8) Numerous clinical trials have shown promising results in primary teeth favoring the use of pulp capping materials in pulpotomy in children. (8,13)

Pulp Capping Materials

Various pulp capping materials are commercially available including the 'standard' calcium hydroxide-based cements (CHC) (2-3,7,9-10,14-24) calcium aluminate and phosphate-based cements, adhesive systems, zinc oxide eugenol, bioactive materials, and calcium silicate-based cements (CSC). (15,20) These materials have gained popularity in a wide array of clinical procedures involving, but not limited to, direct and indirect pulp capping, pulpotomy, root perforation repair, internal and external root resorption, and root-end apicoectomy due to its anti-inflammatory properties, ability to inhibit bacterial growth, biocompatibility to the pulpal cells and formation of a mineralized tissue between the vital pulp and the oral cavity to prevent ingress of microorganisms which are deemed important prerequisites for a successful pulp capping treatment. (4,7,15, 20-21)

Calcium Hydroxide Cements

CHCs were introduced in 1920 and since then have been considered as a 'standard' material for pulp capping. (3,8-10,12,15-19,22) The high alkaline pH is unfavorable for micro-organisms and thus, exerts an antibacterial action. (3,7,11,22,25) It increases the expression of both alkaline phosphatase and bone morphogenetic protein-2 (BMP-2), promoting the formation of calcific barrier. (23) When placed close to the pulp, it releases calcium and hydroxyl ions leading

to influx of these ions towards the pulp through the remaining dentine. (3,8-10,12,15,16-19, 22, 24) This Ca influx triggers the recruitment and proliferation of undifferentiated cells from the pulp (22) and activates stem cells promoting reparative dentine formation, whereas hydroxyl ions exert an antibacterial action by creating unfavorable condition for the survival of micro-organisms. (3,11,25)

The major limitations of CHCs are dimensional instability, high solubility and disintegration over time leading to tunnel defects (93%). (7,12,15-20,22,24) These tunnel defects are viewed as patent channels containing soft tissue inclusions and inflammatory cells which connect the pulp chamber with the overlying pulp capping material. The disadvantage of the tunnel defects is that they may allow microleakage and slow ingress of toxins and microorganisms from the cavosurface margins to the pulp inducing pulpal degeneration and leading to potential dystrophic calcification and pulp necrosis. (19,22) Histologically, it led to cytotoxicity in cell cultures and has shown to induce apoptosis. Also, it is relatively contraindicated in primary dentition due to risk associated with resorption. (22)

Currently pre-mixed one-paste non-setting CH, two-paste self-setting CH, and resin modified CH pulp capping materials are commercially available. (15,22) It is reported that the two-paste system is more widely adopted than the one paste non-setting system. The latter has disadvantages such as high solubility and lack

of setting. (15,22) Light curable resin modified CHC systems exhibited superior physical properties, low water solubility and ease of use. (15,22) The two-paste system has shown increased toxicity to human dental pulp stem cells (HDPSC) when compared to non-setting one paste system due to presence of disalicylate, accelerator and/or plasticizer. Surprisingly, resin containing CH has shown lower cytotoxicity than two-paste CHC. (15)

Dycal, (Dentsply Dental, Tulsa, USA) is a two-paste self-set (22), CH-based pulp capping material containing a base paste and a catalyst paste. The base paste contains the active ingredient disalicylate, whereas the catalyst paste contains calcium hydroxide. The setting time for two-paste system is usually 2-3 minutes. It contains additional ingredients such as zinc stearate (accelerator), zinc oxide (reactant), calcium tungstate or barium sulfate as a radio opacifier. (15) Among its limitations, the modulus of elasticity, compressive and tensile strength is low, and it is highly soluble. The high alkaline pH results in the antibacterial effect, stimulates coagulative necrosis, pulpal inflammation, and cytotoxicity. (4,7,12,15-22)

Due to these clinical and histological limitations, newer materials like CSC, bioactive materials have gained popularity which provides better marginal seal, produces minimal pulpal inflammation, has low solubility, exhibits favorable biocompatibility, and induces dentine-bridge formation. (25) Clinical trials have indicated a higher success rate of CSC-

based pulp capping materials (81% at 4-5 years) than CHC- based pulp capping materials (56% at 4-5 years). (23)

Calcium Silicate Cements

CSC materials are indicated for use in root perforation repair, apexification, pulp capping procedures, root end filling, and pulpotomy. (15,20) CSC, is mostly calcium oxide and silicone dioxide, constituting 70% to 95% of these cements. (21) On mixing, they form silicate hydrate gel and CH by forming dicalcium silicate, tricalcium silicate and aluminate, and tetra calcium aluminoferrite. (26)

However, the mechanism of action of CSCs is not fully understood (27), it is believed to be similar to CHCs (7). It releases CH as a by-product, (7,27) unlike pure CH, which dissolves over time (27) and causes less pulpal inflammation (4). The high alkaline pH enhances the antibacterial activity, shows higher stimulation of odontoblastic activity, (7) and increased release of calcium ions which promotes predictable dentine-bridge formation, thus better hermetic seal formation when compared to CHCs. (28-29)

Mineral Trioxide Aggregate (MTA), the first commercially available CSC, that reacts with water to produce CH and $CaSiO_4$ hydrate gel and forms hard cement. Increasing number of studies show that MTA has now almost replaced CHC as the 'standard' pulp capping material. (30-31) However, the main drawbacks of this material include: long setting time (4 hours), risks of discoloration, high cost and difficult handling. To overcome these limitations, newer CSCs are gaining popularity amongst clinicians. Examples are tricalcium silicate cement like Biodentine and resin calcium silicate cements (RCSC), such as Theracal LC. (17,22) In a systematic review and meta-analysis by Cushley et al. (23), MTA (86%) showed similar success rate for pulp capping as compared to Biodentine (86%) on a one-year clinical follow-up, whereas Biodentine (86%) performed slightly better than MTA (84%) on a 2–3-year follow-up.

Biodentine (Septodont, Saint-Maurdes-Fosses, France) a tricalcium silicate based permanent dentine substitute is employed in numerous dental treatments. It exhibits acceptable biocompatibility and favorable pulpal response. (22,26) It is a powder-liquid material, with an accelerated setting time of 12 minutes. (25) The accelerated setting time is attributed to increased particle size and calcium chloride accelerator. Compared to CHCs, Biodentine shows improved mechanical properties, lower porosity and solubility, no tunnel defects thus lower microleakage and promotion of tertiary dentine formation. (22,25-26) It infiltrates the dentinal tubule to provide micro-mechanical retention and induces target tissue specific functions resulting in reparative dentine formation, thus

pulpal protection. It is believed, these factors may be responsible for absence of postoperative pain and hypersensitivity. The remineralization might be due to the release of Transforming factor beta 1 (TGF-β1) growth factor from pulp cells which has shown to be involved in odontoblastic differentiation after Biodentine application. (26)

Biodentine XP, (Septodont, Saint-Maurdes-Fosses, France) is an auto mix all-in-one cartridge ready to mix. The components, indications of use, and setting time are the same as Biodentine. It was introduced in June 2022.

Resin Calcium Silicate Cements

Theracal LC is a single-paste light activated RCSC pulp capping material. It consists of 45% Portland cement CEM III and 45% composite resin. (17,22) It is 4th generation CSC according to ISO 9917-2017 is a cement 'in which the setting reaction is light activated. It contains fumed silica as thickening agent, barium oxide and barium sulfate as radio opacifiers. Theracal LC is opaque and whitish in color and should be placed in an increment of 1mm followed by light curing for 20 seconds. (17) It has short setting duration, is easy to use, exhibits low solubility and higher compressive strength compared to Dycal and Biodentine. The setting reaction in Theracal LC is by hydration. Theracal LC

does not include water, so it depends on water from the surrounding environment for its diffusion within material. Hence, it is recommended to be placed on moist dentine. (3234) On reacting with water from environment, it releases CH resulting in high pH, explaining its antibacterial nature and releases calcium. It induces poorly formed dentine-bridge at the exposure site with low inflammatory cellular response. (17,32-34) A major limitation of this material is heat generation, inducing adverse pulpal effects in pulp capping procedures, (32) and very low biocompatibility (37) due to presence of resin-based materials. The presence of unpolymerized residual resin component may react with pulpal tissues and leech BisGMA, TEGDMA, UDMA, camphoroquinone, and other heavy trace metals raising concerns of its toxic effects on odontoblastic cells. (32-34)

Antibacterial Activity of Pulp Capping Materials

An effective pulp capping material should protect an exposed pulp from microbial invasion and appropriately eliminate microorganisms and inhibit the bacterial growth to maintain the pulp vitality. (2,4,14-15,24,35) *In vitro (2,14,24,35)* studies have studied the antibacterial effects of different pulp capping, on *S. mutans, a* gram-positive facultative anaerobic cocci , a microorganism frequently associated with initiation of dental caries eventually progressing to pulpal pathology, if left untreated.(10,20,35) On the other hand, *E. faecalis,* a gram-positive facultative anaerobe, is commonly associated with root canal pathology

and secondary infections involving the root canal.(35) Therefore, *S. mutans* and *E. faecalis* were the bacteria included in the study due to their association with dental caries and endodontic infections respectively.

The antibacterial activity of CHCs is dependent on the release of hydroxyl ions in an aqueous medium. (3,10,19,24) The hydroxyl ions are highly oxidative free radicals that rarely diffuse from the site of generation. (4,7,12,15-20,22-24) It is further to be noted that the lethal effects of hydroxyl ions on bacteria is probably because of damage to the bacterial cytoplasmic membrane, denaturation of proteins, or damage to DNA. (5,7,23) The antibacterial activity of the CSCs like Biodentine is similar to that of CHC in which the cements on hydration produce $CaSiO_4$ hydrate gel, CH, and unreacted tricalcium silicate. As discussed earlier, antimicrobial action of CSC is understood. (2)

In the present study, direct culture test (DCT) was used to study the efficacy of bacterial inhibition rather than agar diffusion test (ADT) used for many previous tests. (14,24) The DCT, as described by Weiss *et al*., is a quantitative and

reproducible test to simulate contact of the microorganism with the dental materials (36) whereas ADT allows direct comparison between tested microorganisms and has several shortcomings. (2,36).

Noted of these limitations is ADT's inability to distinguish between bacteriostatic and bactericidal properties of the materials (2). For DCT, the disadvantage is increased contamination risk.(36) The advantage of DCT is that it is less affected by artificial constructs of testing methodology than ADT.(2) In the ADT, the size of the inhibition zones from a certain substance depends on its infusibility in the culture medium used, the contact area between experimental material and agar, molecular weight, size and shape of the antibacterial agent, and ionic concentration of tested material in relation to the medium. (2,35) Furthermore, standardization inoculation, incubation temperature of the plate, selection of agar medium and reading of inhibition zones is responsible for variability in ADT. (2) These issues are the main disadvantages of this semi-quantitative method. (35).

While most of the studies compared antibacterial activity using ADT (14,24) Several studies compared the antimicrobial efficacy by using DCT. Koruyucu *et al*. (35) evaluated the antibacterial activity of Dycal, MTA and Biodentine against *E. faecalis* and found MTA and CSC- based Biodentine had higher bacterial inhibition compared to Dycal. Surprisingly, contradictory results were found by Fathy *et al.* (2) that Biodentine and Theracal LC had no statistical difference on the DCT using *S. mutans.*

However, studies are consistent when using ADT (14,24) which showed the

highest bacterial growth inhibition for CSC compared to CHC. (2,14,24,35) Since CHC are considered a reference material for pulp capping, its inclusion in the current study was necessary. (4,7-8,15-19,22,24) Additionally, no study on the antimicrobial activity of Biodentine XP against S. *mutans* and E. *faecalis* have been reported. Also, extensive studies comparing Dycal, Theracal LC and, Biodentine on ADT have been reported but, to our knowledge, there has been lack of study comparing antibacterial activity of the pulp capping material by DCT. Therefore, comparing the antibacterial activity of Dycal, Theracal LC, Biodentine and, Biodentine XP against *S. mutans* and *E. faecalis* was necessary. there has been no study to our knowledge that studied the biocompatibility of Biodentine XP and all four materials together. Hence, this experiment was necessary and further research was needed.

Biocompatibility of Pulp Capping Materials

A material is said to be a biocompatible pulp capping material when it prevents inflammation, genotoxicity, or carcinogenic action when it contacts the pulp tissue. (15,37) Since the material will be in contact with the pulp for long period

of time, it is necessary to study the biocompatibility of the materials. Various *in vitro* studies (14-15,18,21-22,36-41) and systematic reviews (37) have compared the biocompatibility of different pulp capping materials on HDPSC. Manaspon *et al.* (21) compared the *in vitro* biocompatibility and bioactive properties of ProRoot MTA, Biodentine, Dycal, Theracal LC on HDPSC suggesting superior

biocompatibility of ProRoot MTA and Biodentine compared to Dycal and Theracal LC that showed the highest toxicity. Similarly, Kim *et al. (*22) evaluated the biocompatibility and bioactivity of ProRoot MTA, Biodentine, Theracal LC, and Dycal and concluded ProRoot MTA, Biodentine, and Theracal LC exhibited better biocompatibility and bioactivity when compared to Dycal. Kang *et al*. (18) concluded that Biodentine showed highest biocompatibility compared to ProRoot MTA followed by Theracal LC. Consistent with the previous studies, (18,22,36) Poggio *et al.* compared the biocompatibility of Biodentine, Calcicur, Calcimol LC, Theracal LC, MTA Angelus and Dycal concluding that Biodentine had better biocompatibility than CH- based Dycal (14)

Of particular interest is the study by Chen *et al.* (15) and Nowicka *et al.* (37) on biocompatibility of resin-free and resin-modified pulp capping materials suggesting that light-cured RCSC exhibited higher cytotoxicity than resin-free version probably due to formation of oxygen inhibition layer. Overall, they favored the use of CSC- based materials when compared to CH. (15,38-41). Finally, a study by Bortoluzzi *et al.* concluded that the toxic effects of Bidoentine and Theracal LC were dose and time dependent but favored the use of Biodentine over Theracal LC. (38)

Although all the included studies favored the use of Biodentine compared to Theracal LC and Dycal, so far there has been no study in our knowledge that studied

the biocompatibility of Biodentine XP, and all four materials together. Hence, this experiment was necessary and further research was needed.

Mineralization Potential of Pulp Capping Materials

A pulp capping material should induce mineralization and promote reparative dentine and dentine-bridge formation. The formation of hard tissue promotes hermetic seal thus, preventing microleakage and contributing to pulpal vitality. Various studies (17,22,36) and systematic review (17,27) on mineralization inducing potential of pulp capping materials have been done on HDPSC. Kang *et al*. (18) studied the capacity of ProRoot MTA, Biodentine, and Theracal LC in HDPSC to induce mineralization and observed that Biodentine has the highest mineralization-inducing potential. Inconsistent with the results of previous studies, Kim *et al.* (22) observed that Biodentine showed lower calcified nodule formation compared to the Theracal LC on comparing the calcium nodule formation of ProRoot MTA, Biodentine, Theracal LC, and Dycal. However, under light electron microscopy, formation of complete dentine bridge Biodentine having layers of well-arranged odontoblasts and odontoblast-like cells with no evidence of inflammatory response was observed. However, Manaspon *et al.* (36) compared the *in vitro* properties of ProRoot MTA, Biodentine, Dycal and Theracal LC on HDPSC suggesting that calcium ions are a bioactive ingredient in pulp capping materials. Biodentine showed highest calcium release, and Theracal LC showed more uniform dentine-bridge compare

to Dycal.

In systematic review and meta-analysis, Andrei *et al*. (17) and Pedano *et al*., (25) it was concluded that resin free pulp capping materials showed better dentine-bridge formation when compared to resin containing materials.

Histological diagnosis does not always corelate with clinical diagnosis. In clinical trials by Jalan *et al*. (7) Peskersoy *et al.* (16) and Sahin *et al*. (12) Biodentine showed continuous and uniform dentine-bridge formation on a 6-month recall radiograph when compared to Dycal and Theracal LC. This clinical data confirms histological findings. (11,37-38)

So far there has been no study in our knowledge that studied the biocompatibility of Biodentine XP, and all the four materials together. Hence, this experiment was necessary and further research was needed.

2. OBJECTIVES

General Objective

The main objective of this study is to assess the *in vitro* antibacterial activity, biocompatibility, and mineralization-inducing potential of Dycal®, Theracal LC®, Biodentine®, and Biodentine® XP.

Specific Objectives

1. To evaluate the *in vitro* antibacterial activity of Dycal®, Theracal LC®, Biodentine®, and Biodentine® XP against *S. mutans* and *E. faecalis* respectively using direct culture test.

2. To evaluate the *in vitro* biocompatibility of Dycal®, Theracal LC®, Biodentine®, and Biodentine® XP with odontoblasts.

3. To evaluate the *in vitro* mineralization-inducing potential of Dycal®, Theracal LC®, Biodentine®, and Biodentine® XP in odontoblasts.

3. HYPOTHESES

General Null Hypothesis

There is no difference in the *in vitro* antibacterial activity, biocompatibility, and mineralization-inducing potential of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP.

Specific Null Hypothesis

1. There is no difference in the *in vitro* antibacterial activity of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP against *S. mutans* and *E. faecalis* respectively using direct culture test.

2. There is no difference in the *in vitro* biocompatibility of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP with odontoblasts.

3. There is no difference in the *in vitro* mineralization-inducing potential of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP in odontoblasts.

4. MATERIALS AND METHOD

Materials	Composition	Handling property/pH	Lot No.
Dycal ® (Dentsply Dental, Tulsa, USA)	**BASE PASTE:** 1,3butylene glycol disalicylate, zinc oxide (ZnO), calcium phosphate ($Ca_3PO_4)_2$, calcium tungstate ($CaWO_4$), iron oxide pigments **CATALYST PASTE:** Calcium hydroxide, ethyl toluenesulfonamide, zinc stearate, titanium dioxide, zinc oxide, iron oxide	Hand-mixed Self-cure Two paste system Setting time: 2-3 mins pH- 9-11	00099692

Theracal LC ® (Bisco Inc., Schamburg, USA)	45% wt mineral material (type III Portland cement), 10% wt radiopaque component, 5% w hydrophilic thickening agent (fumed silica) and approximately 45% resin **Resin:** hydrophobic monomers such as urethane dimethacrylate (UDMA), bisphenol A-glycidyl (BisGMA), triethylene glycol dimethacrylate (TEGDMA) and hydrophilic monomers such as hydroxyethyl methacrylate (HEMA), polyethylene glycol dimethacrylate (PEGDMA)	Syringe mixed Light cure Setting time: 20 secs each 1mm increment pH- 10-11	2200004609
Biodentine ® (Septodont, Saint-MaurdesFosses, France)	**POWDER**: tricalcium silicate (C_3S), dicalcium silicate (C_2S), calcium carbonate and oxide, iron oxide, zirconium oxide **LIQUID**: calcium chloride accelerator, hydro-soluble polymer, water, reducing agent	Machine-mixed Self-cure Setting time: 12mins pH- 12	B28892

Biodentine ® XP (Septodont, Saint-MaurdesFosses, France)	**POWDER**: tricalcium silicate (C_3S), dicalcium silicate (C_2S), calcium carbonate and oxide, iron oxide, zirconium oxide **LIQUID**: calcium chloride accelerator, hydro-soluble polymer, water (reducing agent)	Machine-mixed Self-cure Setting time: 12mins pH- 12	B28568AA B28569AA

Table 1. General properties of the pulp capping materials, composition, handling property, setting time, pH and lot number.

Specimen Preparation

Under aseptic conditions, the tested pulp capping materials - Dycal® (Dentsply Dental, Tulsa, OK, USA), Theracal® LC (Bisco Inc., Schamburg, IL), Biodentine® (Septodont, Saint-Maur-de-Fosses, France), and Biodentine® XP (Septodont, Saint-Maur-de-Fosses, France) - were mixed according to the manufacturers' instructions. The mixed materials were then loaded into silicone molds with a diameter of 4 mm and a depth of 2 mm to prepare uniform discs. Once the materials were set, the specimens were unmolded to obtain the final uniform discs.

Bacteria and Culture Conditions

To evaluate the antibacterial effect of the pulp capping materials two types of bacteria, *Streptococcus mutans* UA159 and *Enterococcus faecalis* ATCC 29212 were used in the present study. To prepare the bacteria for the experiment, they were first cultured on agar plates containing Todd Hewitt (TH) and 0.5% yeast extract at 37°C in a 5% CO2 incubator for 48 hours. Single colonies of the bacteria were then inoculated into THY broth and cultured overnight. To measure the number of bacteria, the optical density (OD) at 600 nm was determined using a spectrometer. This allowed for the determination of colony forming unit (CFU) for each bacterial strain.

Direct Culture Test

The pulp capping materials were placed in a 24-well cell culture plate with 1 ml of THY medium and left to incubate at room temperature overnight. Next, a bacterial suspension containing 1 x 107 colony forming units (CFU)/ml and 100 µl volume was added to each well and cultured for 6 hours. Following the 6-hour incubation, the bacterial cultures from each well were diluted ten-fold and cultured on THY agar plates for 48 hours. The number of CFUs for each bacterial strain was then determined. To evaluate the antibacterial effects of each pulp capping material, the CFU counts were compared to those of the positive control. Duplicate experiments were conducted, and a total of three independent experiments were performed.

Cell Culture

To assess the biocompatibility and ability to induce mineralization of the pulp capping materials, the mouse 17IIA11 odontoblast progenitor cell line was utilized. The cells were cultured in standard Dulbecco's modified eagle medium (DMEM) supplemented with 5% fetal bovine serum, 100 µg/ml streptomycin and, 100 units/ml penicillin in a CO2 incubator. For odontoblast differentiation, cells were seeded at a density of 1 x 105 cells/well in 24-well plates with transwell inserts (Costar #3413). Once cells reached 8595% confluency, odontogenic differentiation was induced by adding 7 mM glycerophosphate and 50 µg/ml ascorbic acid. The culture medium was changed every 48 hours. To

evaluate the pulp capping materials, they were prepared as described earlier and placed in the transwell inserts at specified time intervals. Subsequently, the cells were assessed for their biocompatibility and mineralization response to the materials.

3-(4,5-dimethyl thiazolyl-2yl)-2,5-diphenyltetrazolium bromide MTT Assay

To assess the impact of the tested pulp capping materials on preodontoblast viability and proliferation, the 3-(4,5-dimethyl thiazolyl-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed. 17IIA11 preodontoblasts were cultured with the tested pulp capping materials in standard medium within 24-well transwell plates for 24 hours. Following the incubation period, MTT labeling reagent (0.5 mg/ml, Invitrogen Life Technologies, Eugene, Oregon) was added to each well. The cells were then incubated for an additional 4 hours. Subsequently, the supernatants were removed, and the intracellularly stored MTT formazan was dissolved in 200 µL of dimethyl sulfoxide, followed by an additional 4hour incubation. Optical densities (ODs) were measured at 570 nm using a microplate spectrophotometer. Duplicate experiments were conducted, and 3 independent experiments were performed to ensure consistent results. The results of the MTT assay were analyzed to determine the impact of the tested materials on preodontoblast viability and proliferation.

Alizarin Red S (ARS) Staining and Semi-Quantification of Mineralization

To assess the mineralization-inducing potential of the pulp capping materials, cells were cultured with the materials in osteogenic medium for 5 days. Subsequently, the cells were fixed with 4% neutral buffered formalin for a minimum of 1 hour and stained with a 2% ARS (Sigma Aldrich) staining solution (pH 4.1-4.3) in the dark for 45 minutes. Excess dye was removed by washing the cells with distilled water. Calcium deposits were quantified by extracting ARS from the stained cells using 10% cetylpyridinium chloride (CPC) and measuring it via colorimetric detection at OD 570 nm using a microplate spectrophotometer. Duplicate experiments were conducted, and a total of three independent experiments were performed.

Statistical Analysis

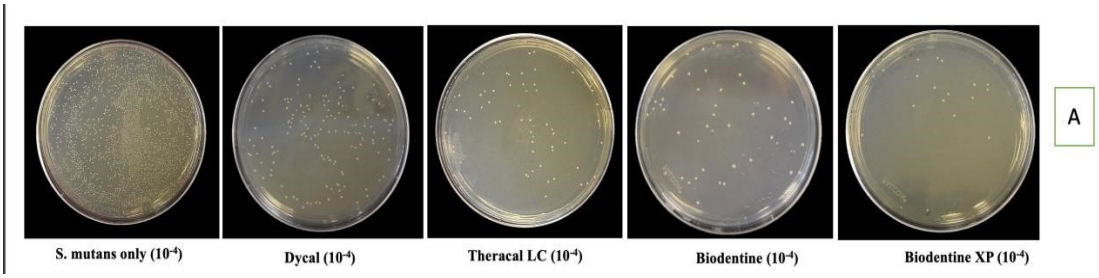
All data were expressed as mean ± SEM (standard error of the mean) and the statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism 9 (GraphPad Software Inc, San Diego, CA). Differences between groups were considered significant at $p<0.05$.

5. RESULTS

Evaluation of the Antibacterial Activity of the Pulp Capping Materials Using the Direct Culture Test

Antibacterial Activity of the Pulp Capping Materials Using the DCT Against *S. mutans*

Of the tested materials, Biodentine XP showed highest bacterial inhibition of *S. mutans* activity when compared to the control ($p<0.0001$). The pulp capping materials showed significant inhibition on *S. mutans* activity. Biodentine ($p<0.0001$) and Theracal LC ($p<0.0001$) showed inhibition of S. mutans. However, Dycal ($p<0.0001$) showed the least inhibition against S. mutans on DCT. (Figure 1)



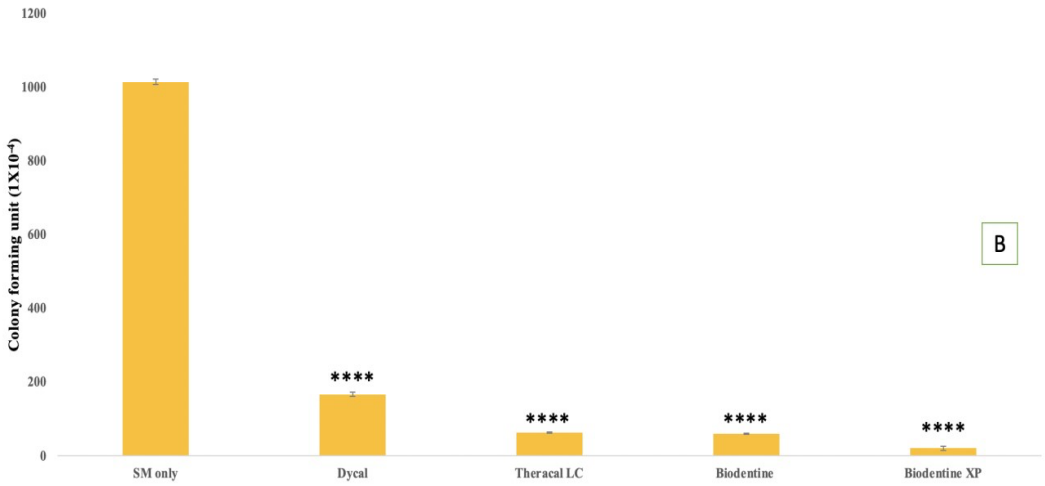


Figure 1. Antibacterial activity against *S. mutans* by DCT. *S. mutans* were cultured alone or with the pulp capping materials for 6 h. Bacterial cultures were ten-fold serially diluted and cultured on THY agar plates for 48 h and colonies were counted. A. Representative images of *S. mutans* colonies on THY agar plates. B. CFUs were hand counted and data are expressed as mean ± SEM (n=6) compared with the *S. mutans* only control. ****$p < 0.0001$

Antibacterial Activity of the Pulp Capping Materials Using the DCT Against E. faecalis

All the pulp capping materials showed *E. faecalis* inhibition when compared to the *E. faecalis* only control. Biodentine XP (p=0.0026) and Biodentine (p=0.0018) showed strongest inhibition compared to *E. faecalis* only control followed by Theracal LC (p=0.0511). Dycal (p=0.1375) showed the least (p= inhibition against *E. faecalis* on DCT. The tested pulp capping materials showed satisfactory antimicrobial activity on *E. faecalis.* (Figure 2)

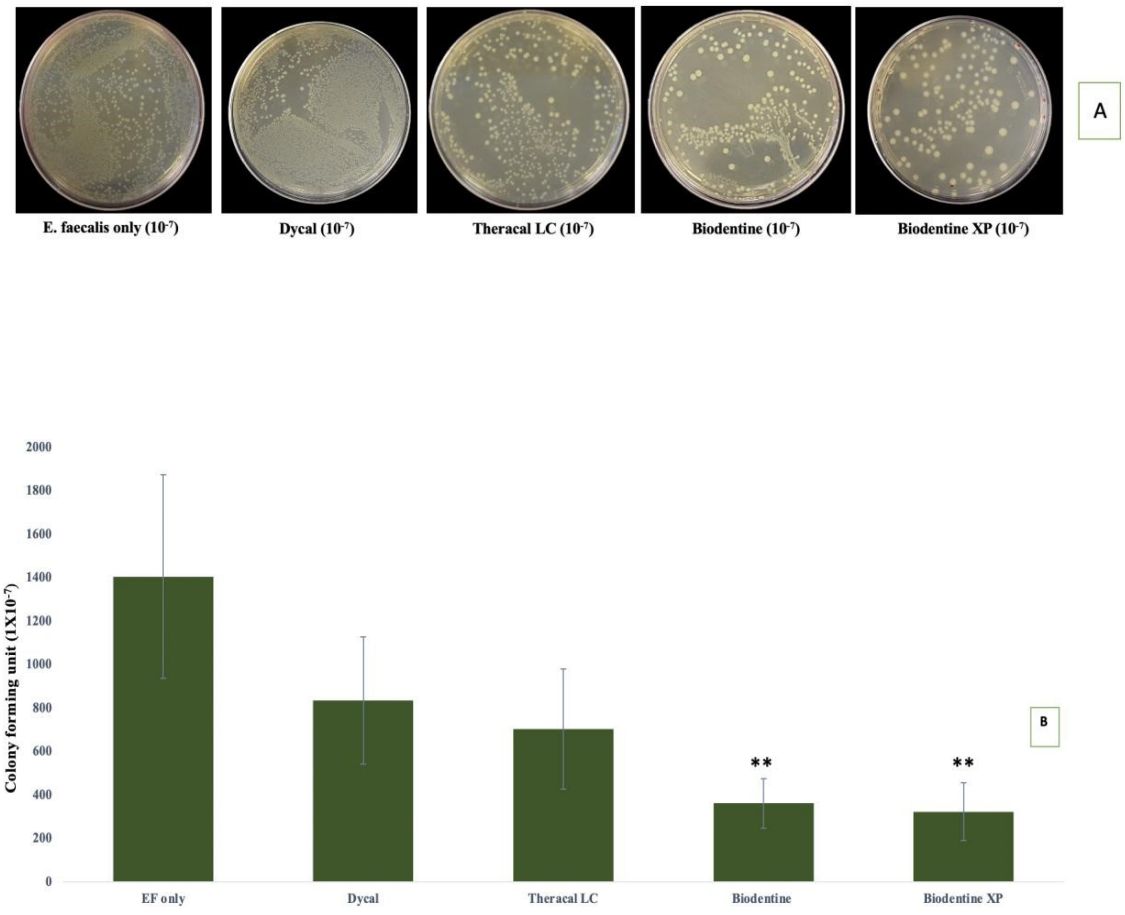


Figure 2. Antibacterial effects against *E. faecalis* by DCT. *E. faecalis* were cultured alone or with the pulp capping materials for 6 h. Bacterial cultures were ten-fold serially diluted and cultured on THY agar plates for 48 h and colonies were counted A. Representative images of *E. faecalis* colonies on THY agar plates. B. CFUs were hand counted, and data are expressed as mean ± SEM (n=6) compared with the *E. faecalis* only control.

Evaluation of the Biocompatibility of the Pulp Capping Materials on MTT Assay

In the following experiment, the viability of the 17IA11 preodontoblast cells was evaluated on MTT Assay following 24 hours incubation. Of the tested materials on MTT Assay, both Biodentine ($p=0.9957$) and Biodentine XP ($p=0.8249$) showed cell viability similar to positive control; whereas statistically significant differences were observed in cell viability of Theracal LC ($p<0.0001$) and Dycal ($p<0.0001$) when compared to positive control. Dycal exhibited the least biocompatibility of all the tested materials. (Figure 3)

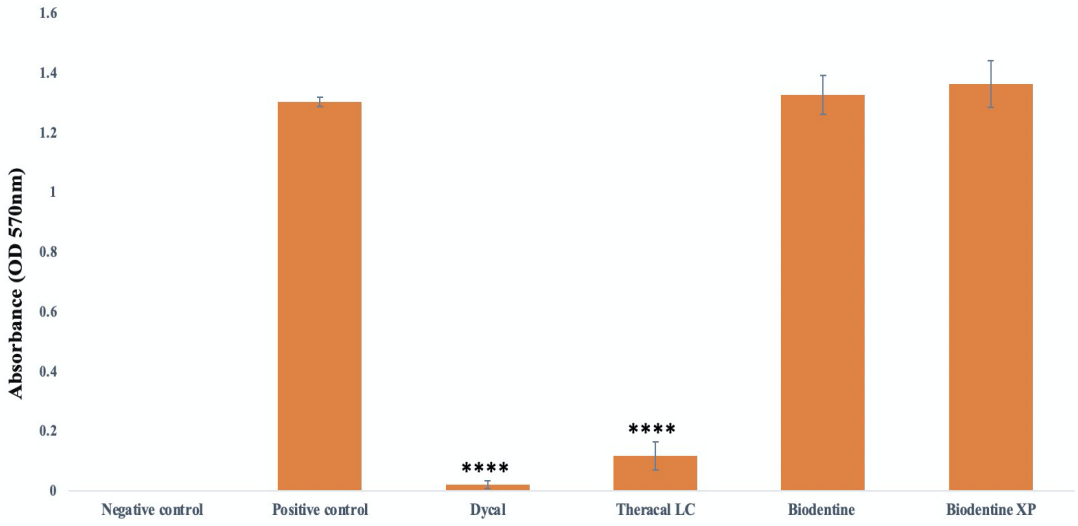
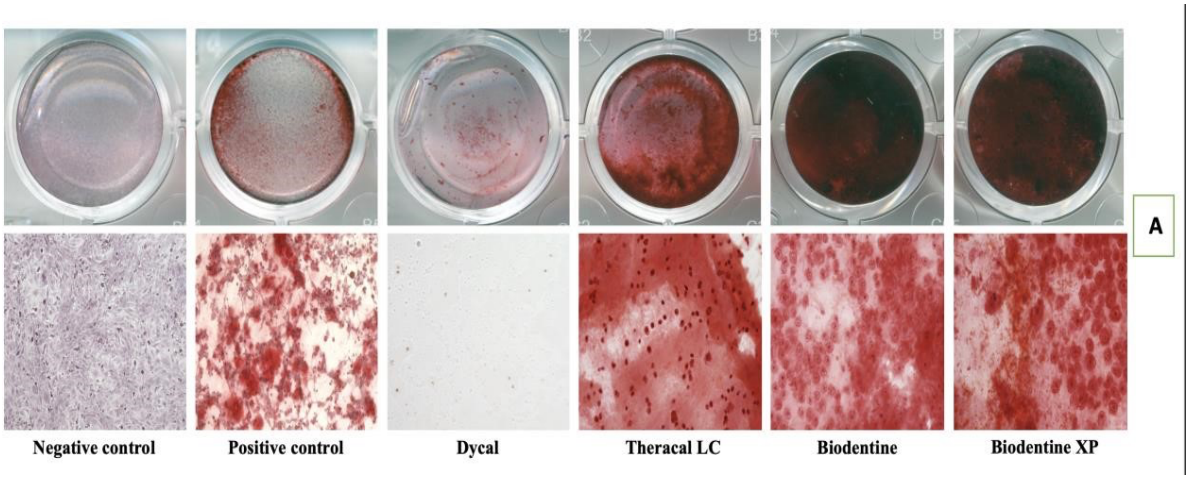


Figure 3. Cytotoxicity of materials on odontoblasts. 17IIA11 preodontoblasts were cultured in the standard medium with or without pulp capping materials for 24 h. Cellular viability was evaluated by measuring absorbance of formazan product at 570 nm using the MTT assay. Data are expressed as mean ± SEM (n=6). ****$p<0.0001$.

Evaluation of the Mineralization of the Pulp Capping Materials on Alizarin Red S (ARS) Staining

17IIA11 cells when cultured in osteogenic medium differentiate into odontoblast-like cells and the differentiated odontoblasts feature expansive extracellular calcium deposits that can be stained using ARS stain. The effect of different materials on mineralization was evaluated using ARS staining on day 5. On day 5 of the ARS test, cultures with Biodentine XP, Biodentine and Theracal LC showed distinct mineralized nodules compared to control but weren't statistically different. However, Dycal ($p<0.0001$) significantly inhibited odontoblast mineralization. (Figure 4)



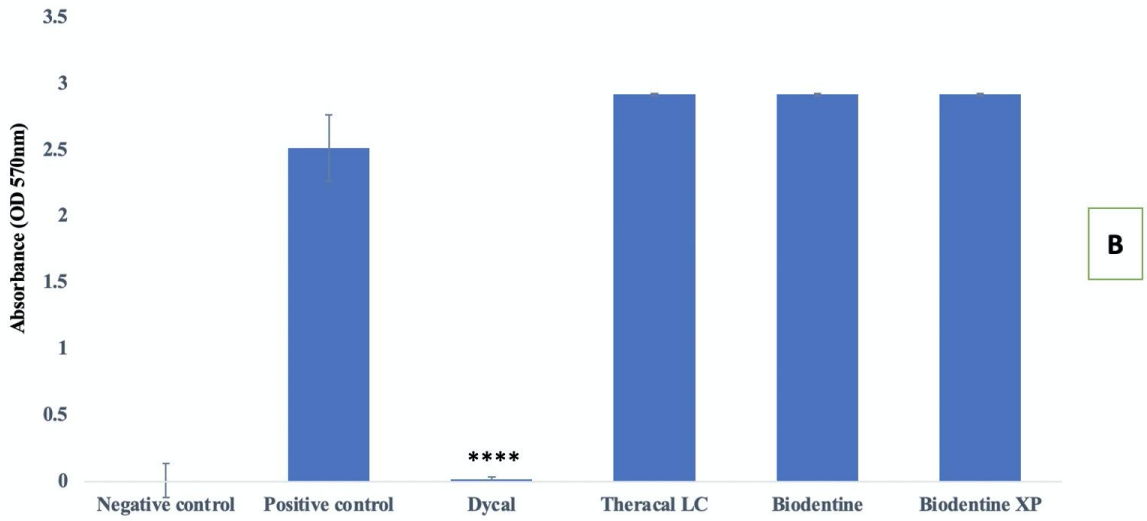


Figure 4. Effects of materials on the mineralization of odontoblasts. 17IIA11 preodontoblasts were cultured in the osteogenic medium with or without pulp capping materials for 5 days. Cells cultured in the standard medium served as negative controls. ARS staining was used for evaluation of mineralization inducing potential. A. Representative images of ARS staining. B. Quantification of ARS staining. Data are expressed as mean ± SEM (n=6). ****$p<0.0001$

6. DISCUSSION

Preservation of the pulp is very important for various reasons like maintaining pulp vitality by inhibiting bacterial growth, show acceptable biocompatibility and induce hard tissue formation by pulpal cells. (16) In our research, we evaluated the *in vitro* antibacterial activity, biocompatibility and mineralization inducing potential of four pulp capping materials, namely, Dycal, Theracal LC, Biodentine and Biodentine XP.

The antibacterial activity is a desirable property of pulp capping materials, as the prevention of bacterial penetration will reduce the risk of endodontic infections. (14) In the present study, DCT was used for evaluating the antibacterial effect of the tested pulp capping materials. All the tested pulp capping materials showed inhibition against *S. mutans* and *E. faecalis* with stronger predictable results against *S. mutans* than *E. faecalis.* Consistent with the previous studies, CSC based materials like Biodentine showed highest inhibition against *S. mutans.* (2)

In our study, we found that CSC based cements Biodentine XP and Biodentine were superior in inhibiting the growth of *E. faecalis* and *S. mutans.* We also found that Theracal LC showed some inhibition of *E. faecalis,* but Dycal was the least effective in preventing bacterial growth.

The antibacterial activity CHC, Dycal is due to its high alkalinity and release of hydroxyl ions creating unfavorable conditions for organisms and thus leading to antibacterial action by acting on the cytoplasmic membrane. (2,5,23-24) However, consistent with the previous study (42) the antibacterial activity of CSC was superior to RCSC, due to higher release of CH in Biodentine compared to Theracal LC. Also, pulp capping materials may not be effective against many microorganism strains, so disinfection using chlorhexidine or bleach is necessary prior to the placement of the medicament for improved treatment outcomes. (43)

Biocompatibility is an important characteristic of pulp capping material as these pulp capping materials will continue to be in direct contact with the pulp cells for a long period of time. These materials must be non-toxic to pulp cells since they will induce osteogenic differentiation. (15,22,37) Based on the outcomes of the present research, the tested pulp capping materials showed acceptable biocompatibility. Consistent with the previous studies, (22,20,36) the CSC based materials like Biodentine showed higher biocompatibility than Theracal LC and

Dycal on HDPSC. In a study on rat bone marrow (2) and mouse odontoblast-like cell line (14) Biodentine outperformed Theracal LC and Dycal.

It is believed that the cytotoxicity of the Dycal is due to increased pH which increases the release of hydroxyl ions leading to increased cellular apoptosis due to disruption of the membrane morphology (36, 44, 45); whereas, for Theracal LC cytotoxicity may be reported due to remaining unpolymerized resins monomers like Bis-GMA (2,32-34,39), but it was reported to be avoided with appropriate light curing techniques. It is believed that Bis-GMA may accumulate overtime and cause cytotoxicity since the resin composition can alter lipid layer in the cell membrane. (2,32-34,39) Another reason for low cellular viability may be due to release of camphoroquinone and ethyl-4- (dimethyl amino) benzoate. Camphoroquinone on reaction with human dental pulp fibroblasts produces increased reactive oxygen species. Accounting together, the release of light curing additives may induce cell death due to increase in the reactive oxygen species. (15,38,39-41) For CSC like Biodentine, it has been suggested that the presence of dicalcium and tricalcium silicate are key factors in favoring its biocompatible nature. The high calcium ion release explains its bioactivity, and an increased uptake of silicone from the surrounding dentine contributes to biocompatibility of Biodentine. (46-47) Another reason for biocompatible nature of Biodentine is that it lacked strontium, aluminum and sulfur that are believed to cause cytotoxicity. (8) In previous studies, Biodentine showed increased TGF-

1 activity from pulp cells which induces angiogenesis, cell differentiation, and mineralization. (48)

In this study, we analyzed the mineralization ability of Dycal, Theracal LC, Biodentine and Biodentine XP. In our research experiment, we found that Biodentine XP, Biodentine and Theracal LC showed mineralization on day 5 of the ARS Staining whereas Dycal showed the least mineralization. A pulp capping material should induce mineralization and promote reparative dentine and dentine-bridge formation. The formation of hard tissue promotes hermetic seal thus, preventing microleakage and maintaining the vitality of the pulp. Calcium ions are considered as a bioactive ingredient in pulp capping materials. (49) Almost all previous studies have reported that CSC based cements have shown better mineralization potential than both CHC based Dycal and RCSC based Theracal LC. (12,49)

In the present study, consistent with the outcomes of the previous studies, CHC based Dycal (12,49) showed the least mineralization inducing potential. The formation of dentine-bridge is dependent on the pH of the CHC based material and the amount of calcium and hydroxide released. In lower pH materials like Dycal, necrotic zone formation is seen adjacent to the material but is resorbed before the mineralization process leading to tunnel defects. (6) The mineralization inducing potential of Biodentine is reported to be due to presence of tricalcium

silicates which enhance the proliferation, differentiation, and mineralization of HDPSC due to release of silicone. It is reported to promote osteoblast proliferation and gene expression by involving in metabolism, collagen synthesis, connective tissue crosslinking, and bone mineralization explaining a thick homogeneous dentine-bridge. (47)

In our experiment we found that Theracal LC showed mineralization similar to Biodentine and Biodentine XP. Our results are consistent with a study from Kim *et al.* (18) on HDPSC where mineralization inducing potential of Theracal LC was found to be comparable to Biodentine. On the other hand, other studies reported opposite of the findings where Biodentine performed better than Theracal LC. (2,49) Interestingly, we found Theracal LC showed cytotoxicity, but showed mineralization similar to Biodentine and Biodentine XP. Theracal LC as discussed in Table 1, is predominantly Portland cement and resins. Portland cement is composed of: tricalcium silicate, dicalcium silicate, tricalcium aluminate, and a tetra-calcium aluminoferrite. It is possible that cytotoxicity for Theracal LC may be induced by initial release of additives from camphoroquinone and ethyl-4- (dimethyl amino) benzoate that may have killed many of the cells but there may have been some remaining cells able to produce calcium nodules in response to calcium silicates present. Also, in our study biocompatibility was evaluated on 24 h duration whereas mineralization-inducing potential was evaluated on day 5. However, it is unclear if this discrepancy was due to differences in the duration of the incubation period of

MTT Assay and ARS staining, the cells used, culture conditions or dose of the material used. More *in vitro* studies are necessary to further evaluate the mineralization inducing potential of Theracal LC.

Due to relative newness of the CSC based Biodentine XP and absence of literature on its antibacterial activity, biocompatibility, and mineralization-inducing potential, it is suggested that more *in vitro* and *in vivo* studies are needed. Depending on the results of our experimental set up, and success of Biodentine, it can be concluded that the newer material Biodentine XP showed promising antibacterial activity, biocompatibility, and

7. CONCLUSION

Hypotheses

1. There is no difference in the antibacterial activity of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP against *S. mutans* and *E. faecalis* respectively using direct culture test. - ***Reject***

2. There is no difference in the biocompatibility of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP with odontoblasts. – ***Reject***

3. There is no difference in the mineralization-inducing potential of Dycal®, Theracal LC®, Biodentine® and Biodentine® XP in odontoblasts. - ***Reject***

7.2 Based on the outcomes of the present research, following inferences can be concluded:

1. All the tested pulp capping materials, have anti-bacterial efficacy against *S. mutans* and *E. faecalis*, and Biodentine XP has the highest antibacterial activity.

2. Biodentine XP and Biodentine showed favorable biocompatibility with preodontoblast cells of all the tested pulp capping materials.

3. Biodentine XP, Biodentine, and Theracal LC have the most mineralization potential.

4. Resin free materials are better than resin-containing materials in *in vitro* antimicrobial and biocompatible activities.

5. The newer material Biodentine XP showed excellent antibacterial activity, biocompatibility and mineralization-inducing potential and can be employed in clinical use, but more *in vivo* and *in vitro* studies are needed.

8. REFERENCES

1. AAE – American Association of Endodontists (2016) Glossary of terms.
2. Poggio C, Arciola CR, Beltrami R, Monaco A, Dagna A, Lombardini M, Visai L. Cytocompatibility and antibacterial properties of capping materials. ScientificWorldJournal. 2014; 2014:181945. doi: 10.1155/2014/181945. Epub 2014 May 18. PMID: 24959601; PMCID: PMC4052471.
3. Fuks AB. Current concepts in vital primary pulp therapy. Eur J Paediatr Dent. 2002 Sep;3(3):115-20. PMID: 12870999.
4. Brizuela C, Ormeño A, Cabrera C, Cabezas R, Silva CI, Ramírez V, Mercade M. Direct Pulp Capping with Calcium Hydroxide, Mineral Trioxide Aggregate, and Biodentine in Permanent Young Teeth with Caries: A Randomized Clinical Trial. J Endod. 2017 Nov;43(11):1776-1780. doi: 10.1016/j.joen.2017.06.031. Epub 2017 Sep 14. PMID: 28917577.
5. Mohammadi Z, Shalavi S, Yazdizadeh M. Antimicrobial activity of calcium hydroxide in endodontics: a review. Chonnam Med J. 2012 Dec;48(3):133-40. doi: 10.4068/cmj.2012.48.3.133. Epub 2012 Dec 21. PMID: 23323217; PMCID: PMC3539092.
6. Haskell EW, Stanley HR, Chellemi J, Stringfellow H. Direct pulp capping treatment: a long-term follow-up. J Am Dent Assoc. 1978 Oct;97(4):607-12. doi: 10.14219/jada.archive.1978.0356. PMID: 281402.

7. Jalan AL, Warhadpande MM, Dakshindas DM. A comparison of human dental pulp response to calcium hydroxide and Biodentine as direct pulp-capping agents. J Conserv Dent. 2017 Mar-Apr;20(2):129-133. doi: 10.4103/09720707.212247. PMID: 28855762; PMCID: PMC5564240.
8. Ghoddusi J, Forghani M, Parisay I. New approaches in vital pulp therapy in permanent teeth. Iran Endod J. 2014 Winter;9(1):15-22. Epub 2013 Dec 24. PMID: 24396371; PMCID: PMC3881297.
9. Casamassimo P, Nowak A. The handbook of pediatric dentistry. 5th. American Academy of Pediatric Dentists; 2018
10. Paula AB, Laranjo M, Marto CM, Paulo S, Abrantes AM, Casalta-Lopes J, Marques-Ferreira M, Botelho MF, Carrilho E. Direct Pulp Capping: What is the Most Effective Therapy?-Systematic Review and Meta-Analysis. J Evid Based Dent Pract. 2018 Dec;18(4):298-314. doi: 10.1016/j.jebdp.2018.02.002. Epub 2018 Feb 15. PMID: 30514444.
11. Tuna D, Olmez A. Clinical long-term evaluation of MTA as a direct pulp capping material in primary teeth. Int Endod J. 2008 Apr;41(4):273-8. doi: 10.1111/j.1365-2591.2007.01339.x. Epub 2007 Nov 27. PMID: 18042222.
12. Sahin N, Saygili S, Akcay M. Clinical, radiographic, and histological evaluation of three different pulp-capping materials in indirect pulp treatment of primary teeth: a randomized clinical trial. Clin Oral Investig. 2021 Jun;25(6):3945-3955. doi: 10.1007/s00784-020-03724-4. Epub 2021 Jan 6. PMID: 33404764.
13. American Academy of Pediatric Dentistry. American Academy of Pediatric Dentistry reference manual. American Academy of Pediatric Dentistry; 2009

14. Poggio C, Arciola CR, Beltrami R, Monaco A, Dagna A, Lombardini M, Visai L. Cytocompatibility and antibacterial properties of capping materials. ScientificWorldJournal. 2014;2014:181945. doi: 10.1155/2014/181945. Epub 2014 May 18. PMID: 24959601; PMCID: PMC4052471.
15. Chen L, Suh BI. Cytotoxicity and biocompatibility of resin-free and resinmodified direct pulp capping materials: A state-of-the-art review. Dent Mater J. 2017 Jan 31;36(1):1-7. doi: 10.4012/dmj.2016-107. Epub 2016 Dec 6. PMID: 27928102.
16. Peskersoy C, Lukarcanin J, Turkun M. Efficacy of different calcium silicate materials as pulp-capping agents: Randomized clinical trial. J Dent Sci. 2021 Mar;16(2):723-731. doi: 10.1016/j.jds.2020.08.016. Epub 2020 Sep 17. PMID: 33854725; PMCID: PMC8025185.
17. Andrei M, Vacaru RP, Coricovac A, Ilinca R, Didilescu AC, Demetrescu I. The Effect of Calcium-Silicate Cements on Reparative Dentinogenesis Following Direct Pulp Capping on Animal Models. Molecules. 2021 May 6;26(9):2725. doi: 10.3390/molecules26092725. PMID: 34066444; PMCID: PMC8125639.
18. Kang S. Mineralization-inducing potentials of calcium silicate-based pulp capping materials in human dental pulp cells. Yeungnam Univ J Med. 2020 Jul;37(3):217-225. doi: 10.12701/yujm.2020.00248. Epub 2020 May 22. PMID: 32438533; PMCID: PMC7384909.
19. Cox CF, Sübay RK, Ostro E, Suzuki S, Suzuki SH. Tunnel defects in dentin bridges: their formation following direct pulp capping. Oper Dent. 1996 Jan-Feb;21(1):4-11. PMID: 8957909.

20. Chang SW, Lee SY, Ann HJ, Kum KY, Kim EC. Effects of calcium silicate endodontic cements on biocompatibility and mineralization-inducing potentials in human dental pulp cells. J Endod. 2014 Aug;40(8):1194-200. doi: 10.1016/j.joen.2014.01.001. Epub 2014 Feb 12. PMID: 25069932.
21. Manaspon C, Jongwannasiri C, Chumprasert S, Sa-Ard-Iam N, Mahanonda R, Pavasant P, Porntaveetus T, Osathanon T. Human dental pulp stem cell responses to different dental pulp capping materials. BMC Oral Health. 2021 Apr 26;21(1):209. doi: 10.1186/s12903-021-01544-w. PMID: 33902558; PMCID: PMC8074430.
22. Kim Y, Lee D, Song D, Kim HM, Kim SY. Biocompatibility and Bioactivity of Set Direct Pulp Capping Materials on Human Dental Pulp Stem Cells. Materials (Basel). 2020 Sep 4;13(18):3925. doi: 10.3390/ma13183925. PMID: 32899877; PMCID: PMC7558537.
23. Cushley S, Duncan HF, Lappin MJ, Chua P, Elamin AD, Clarke M, El-Karim IA. Efficacy of direct pulp capping for management of cariously exposed pulps in permanent teeth: a systematic review and meta-analysis. Int Endod J. 2021 Apr;54(4):556-571. doi: 10.1111/iej.13449. Epub 2020 Dec 28. PMID: 33222178.
24. Poggio C, Beltrami R, Colombo M, Ceci M, Dagna A, Chiesa M. In vitro antibacterial activity of different pulp capping materials. J Clin Exp Dent. 2015 Dec 1;7(5):e584-8. doi: 10.4317/jced.52401. PMID: 26644833; PMCID: PMC4663059.
25. Pedano MS, Li X, Yoshihara K, Landuyt KV, Van Meerbeek B. Cytotoxicity and

Bioactivity of Dental Pulp-Capping Agents towards Human Tooth-Pulp Cells: A Systematic Review of In-Vitro Studies and Meta-Analysis of Randomized and Controlled Clinical Trials. Materials (Basel). 2020 Jun 12;13(12):2670. doi: 10.3390/ma13122670. PMID: 32545425; PMCID: PMC7345102.

26. Arandi NZ, Rabi T. TheraCal LC: From Biochemical and Bioactive Properties to Clinical Applications. Int J Dent. 2018 Mar 26;2018:3484653. doi: 10.1155/2018/3484653. PMID: 29785184; PMCID: PMC5892295.
27. Min KS, Park HJ, Lee SK, Park SH, Hong CU, Kim HW, Lee HH, Kim EC. Effect of mineral trioxide aggregate on dentin bridge formation and expression of dentin sialoprotein and heme oxygenase-1 in human dental pulp. J Endod. 2008 Jun;34(6):666-70. doi: 10.1016/j.joen.2008.03.009. Epub 2008 Apr 25. PMID: 18498885.
28. Nair PN, Duncan HF, Pitt Ford TR, Luder HU. Histological, ultrastructural, and quantitative investigations on the response of healthy human pulps to experimental capping with Mineral Trioxide Aggregate: a randomized controlled trial. 2008. Int Endod J. 2009 May;42(5):422-44. doi: 10.1111/j.13652591.2009.01558.x. PMID: 19356180.
29. Ferk Luketić S, Malcić A, Jukić S, Anić I, Segović S, Kalenić S. Coronal microleakage of two root-end filling materials using a polymicrobial marker. J Endod. 2008 Feb;34(2):201-3. doi: 10.1016/j.joen.2007.09.019. PMID: 18215682.

30. Komabayashi T, Zhu Q, Eberhart R, Imai Y. Current status of direct pulp-capping materials for permanent teeth. Dent Mater J. 2016;35(1):1-12. doi: 10.4012/dmj.2015-013. PMID: 26830819.

31. Cuadros-Fernández C, Lorente Rodríguez AI, Sáez-Martínez S, GarcíaBinimelis J, About I, Mercadé M. Short-term treatment outcome of pulpotomies in primary molars using mineral trioxide aggregate and Biodentine: a randomized clinical trial. Clin Oral Investig. 2016 Sep;20(7):1639-45. doi: 10.1007/s00784015-1656-4. Epub 2015 Nov 18. PMID: 26578117.

32. Kunert M, Lukomska-Szymanska M. Bio-Inductive Materials in Direct and Indirect Pulp Capping-A Review Article. Materials (Basel). 2020 Mar 7;13(5):1204. doi: 10.3390/ma13051204. PMID: 32155997; PMCID: PMC7085085.

33. Gandolfi MG, Siboni F, Prati C. Chemical-physical properties of TheraCal, a novel light-curable MTA-like material for pulp capping. Int Endod J. 2012 Jun;45(6):571-9. doi: 10.1111/j.1365-2591.2012.02013.x. Epub 2012 Mar 31. PMID: 22469093.

34. Voicu G, Didilescu AC, Stoian AB, Dumitriu C, Greabu M, Andrei M. Mineralogical and Microstructural Characteristics of Two Dental Pulp Capping Materials. Materials (Basel). 2019 May 31;12(11):1772. doi: 10.3390/ma12111772. PMID: 31159182; PMCID: PMC6600720.

35. Koruyucu M, Topcuoglu N, Tuna EB, Ozel S, Gencay K, Kulekci G, Seymen F. An assessment of antibacterial activity of three pulp capping materials on

Enterococcus faecalis by a direct contact test: An in vitro study. Eur J Dent. 2015 Apr-Jun;9(2):240-245. doi: 10.4103/1305-7456.156837. PMID: 26038657; PMCID: PMC4439853.

36. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod Dent Traumatol. 1996 Aug;12(4):179-84. doi: 10.1111/j.1600-9657.1996.tb00511.x. PMID: 9028181.

37. Nowicka A, Łagocka R, Lipski M, Parafiniuk M, Grocholewicz K, Sobolewska E, Witek A, Buczkowska-Radlińska J. Clinical and Histological Evaluation of Direct Pulp Capping on Human Pulp Tissue Using a Dentin Adhesive System. Biomed Res Int. 2016;2016:2591273. doi: 10.1155/2016/2591273. Epub 2016 Oct 10. PMID: 27803922; PMCID: PMC5075585.

38. Bortoluzzi EA, Niu LN, Palani CD, El-Awady AR, Hammond BD, Pei DD, Tian FC, Cutler CW, Pashley DH, Tay FR. Cytotoxicity and osteogenic potential of silicate calcium cements as potential protective materials for pulpal revascularization. Dent Mater. 2015 Dec;31(12):1510-22. doi: 10.1016/j.dental.2015.09.020. Epub 2015 Oct 19. PMID: 26494267; PMCID: PMC4974822.

39. Nilsen BW, Jensen E, Örtengren U, Michelsen VB. Analysis of organic components in resin-modified pulp capping materials: critical considerations. Eur J Oral Sci. 2017 Jun;125(3):183-194. doi: 10.1111/eos.12347. Epub 2017 Apr 26. PMID: 28444854.

40. Engelmann J, Volk J, Leyhausen G, Geurtsen W. ROS formation and glutathione levels in human oral fibroblasts exposed to TEGDMA and camphorquinone. J

Biomed Mater Res B Appl Biomater. 2005 Nov;75(2):272-6. doi: 10.1002/jbm.b.30360. PMID: 16080163.

41. Tomás-Catalá CJ, Collado-González M, García-Bernal D, Oñate-Sánchez RE, Forner L, Llena C, Lozano A, Moraleda JM, Rodríguez-Lozano FJ. Biocompatibility of New Pulp-capping Materials NeoMTA Plus, MTA Repair HP, and Biodentine on Human Dental Pulp Stem Cells. J Endod. 2018 Jan;44(1):126-132. doi: 10.1016/j.joen.2017.07.017. Epub 2017 Nov 1. PMID: 29079052.
42. Camilleri J. Hydration characteristics of Biodentine and Theracal used as pulp capping materials. Dent Mater. 2014 Jul;30(7):709-15. doi: 10.1016/j.dental.2014.03.012. Epub 2014 Apr 29. PMID: 24793199.
43. Jardine AP, Montagner F, Quintana RM, Zaccara IM, Kopper PMP. Antimicrobial effect of bioceramic cements on multispecies microcosm biofilm: a confocal laser microscopy study. Clin Oral Investig. 2019 Mar;23(3):13671372. doi: 10.1007/s00784-018-2551-6. Epub 2018 Jul 18. PMID: 30022269.
44. Poggio C, Ceci M, Dagna A, Beltrami R, Colombo M, Chiesa M. In vitro cytotoxicity evaluation of different pulp capping materials: a comparative study. Arh Hig Rada Toksikol. 2015 Sep 1;66(3):181-8. doi: 10.1515/aiht-2015-662589. PMID: 26444338.
45. Arandi NZ, Rabi T. TheraCal LC: From Biochemical and Bioactive Properties to Clinical Applications. Int J Dent. 2018 Mar 26;2018:3484653. doi: 10.1155/2018/3484653. PMID: 29785184; PMCID: PMC5892295.
46. Tomás-Catalá CJ, Collado-González M, García-Bernal D, Oñate-Sánchez RE,

Forner L, Llena C, Lozano A, Moraleda JM, Rodríguez-Lozano FJ. Biocompatibility of New Pulp-capping Materials NeoMTA Plus, MTA Repair HP, and Biodentine on Human Dental Pulp Stem Cells. J Endod. 2018 Jan;44(1):126-132. doi: 10.1016/j.joen.2017.07.017. Epub 2017 Nov 1. PMID: 29079052.

47. Camilleri J. The chemical composition of mineral trioxide aggregate. J Conserv Dent. 2008 Oct;11(4):141-3. doi: 10.4103/0972-0707.48834. PMID: 20351970; PMCID: PMC2843533.

48. Laurent P, Camps J, About I. Biodentine(TM) induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012 May;45(5):439-48. doi: 10.1111/j.1365-2591.2011.01995.x. Epub 2011 Dec 22. PMID: 22188368.

49. Laurent P, Camps J, About I. Biodentine(TM) induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012 May;45(5):439-48. doi: 10.1111/j.1365-2591.2011.01995.x. Epub 2011 Dec 22. PMID: 22188368.