

**Effect of Overlying Material on Biodentine™ Setting Reaction in
Primary Molar Pulpotomies**

BY

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THESIS

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LIST OF ABBREVIATIONS

AAPD	American Academy of Pediatric Dentistry
ANOVA	Analysis of Variance
HK	Knoop Hardness
IRM	Interim Restorative Material
RMGI	Resin Modified Glass Ionomer
SSC	Stainless Steel Crown
ZOE	Zinc Oxide Eugenol

SUMMARY

Objective: The purpose of this *in vitro* study was to evaluate the effect of different overlying materials, such as Intermediate Restorative Material (IRM) or resin-modified glass ionomer cement (RMGIC) on the hardness of Biodentine™, used as a pulpotomy agent in primary teeth, as a function of its hardness 24 hours after final tooth restoration. **Methods:** Forty extracted primary molars were mounted in stone. The teeth were randomly selected into four groups of ten teeth each. Occlusal cavities were prepared to the furcation and pulpal debris was excavated in each sample. Group 1 was restored with Biodentine™, IRM, and a Stainless Steel Crown (SSC). Group 2 was restored with Biodentine™, Fuji II, and a SSC. Group 3 was restored with Biodentine™ and an SSC. Group 4 was restored with Biodentine™ only. All samples were stored in an incubator at 37°C and 100% humidity. After 24 hours, samples were sectioned mesio-distally and polished. The setting reaction was measured as a function of Knoop Hardness value (HK) using Leco Microhardness Tester. Each sample was measured at three zones with a 50gf load force for 30 seconds starting at one millimeter from the Biodentine™-material interface. The data was analyzed using One-Way ANOVA and post-hoc Tukey test.

Results: There was no significant statistical difference in the mean value among the four groups ($p>0.05$), and among the three zones. **Conclusions:** In this *in vitro* study, Biodentine™ was a suitable restorative material for primary molar pulpotomies and the overlying material showed no influence on the hardness of Biodentine™ after 24 hours.

1 INTRODUCTION

1.1 Background

According to the American Academy of Pediatric Dentistry (AAPD) guidelines, a primary tooth pulpotomy involves the amputation of the coronal pulp followed by the treatment of vital radicular pulp tissue with medicaments such as Buckley's formocresol solution, ferric sulfate, calcium hydroxide, and mineral trioxide aggregate (MTA)¹. Current pulpotomy agents present different advantages and disadvantages. Buckley's Formocresol solution was introduced in 1904 and has since become the most popular pulp medicament due to its high success rates². However, concerns that formacresol has possible carcinogenic and mutagenic effects propelled a search for an alternative pulpotomy agent²⁻⁴. MTA, which was introduced in 1993, is a biocompatible calcium silicate material that stimulates odontoblastic activity leading to secondary dentin formation³⁻⁵.

Biodentine™ (Septodont, Saint Maur des Fosses, Île-de-France, France) is a new calcium silicate material that also stimulates secondary dentin formation^{3,4}. Studies have revealed that there is no difference in success rates of Biodentine™ and MTA^{3,5}. Compared to MTA, however, Biodentine™ has higher compressive strength, lower porosity, and better color stability⁵⁻⁷. Despite the many desirable characteristics of Biodentine™, the long set time of the material remains an obstacle to its wide use in pediatric dentistry, a field in which working time relies heavily on patient's cooperation.

The manufacturer recommends that the Biodentine™ pulpotomy and the definitive restoration be completed in two visits due to the long set time.

A recent study has examined the placement of a definitive restoration on unset Biodentine™ with plastic teeth⁸. The study found that Biodentine™ displacement was minimal and that it is acceptable to place the definitive restoration three minutes after the Biodentine™ was mixed and placed⁸. Currently no similar evidence exists for natural teeth.

2 REVIEW OF LITERATURE

2.1 History of Pulpotomy Agents

The ideal pulpotomy medicament should be bactericidal, biocompatible, cost effective, promote pulpal healing, have easy handling, and should not interfere with physiologic root resorption⁹⁻¹¹. Currently recognized pulpotomy agents include Buckley's formocresol solution, ferric sulfate, calcium hydroxide, and MTA. Each of these agents presents unique advantages and disadvantages.

2.1.1 Formocresol

Buckley's formocresol solution was introduced in 1904 for treatment of non-vital permanent teeth^{2,10}. In 1930, formocresol was introduced as a pulpotomy medicament for primary teeth, consisting of 19% formaldehyde and 35% cresol in glycerin or water¹⁰. Formocresol remains a popular pulpal medicament today due to its high success rate of 89.6%^{10,12}. While acknowledging formocresol's high success rate, it must also be mentioned that formaldehyde is classified as a probable carcinogen by the U.S. Department of Health and Human Services and the U.S. Environmental Protection Agency¹². A study measuring formocresol and cresol levels in blood plasma of children before and after pulpotomy treatment under general anesthesia found that formaldehyde levels were undetectable above baseline plasma concentration and cresol levels were undetectable¹³. Despite the limited evidence that formocresol used in dental treatment poses a risk to patient health, the concerns of potential mutagenicity are enough to prevent it from being accepted as an ideal agent and has propelled a search for an alternative pulpotomy agent^{2,3,10}.

2.1.2 Glutaraldehyde

Glutaraldehyde was introduced as a potential replacement for formocresol in endodontic procedures by s'Gravenmade in 1975 and then in primary teeth pulpotomies by Kopel and colleagues. in 1980^{9,14}. Glutaraldehyde is an attractive agent due to its superior fixative properties and its self-limiting penetration that leads to low antigenicity and low toxicity⁹. Glutaraldehyde is effective as a bactericidal agent at a pH of 7.5 to 8.5⁹. However, in its effective form, glutaraldehyde is unstable and has a shelf life of two weeks⁹. Therefore, the practicality of glutaraldehyde as a pulpotomy agent is limited by its short shelf life despite an average success rate of 82 to 95 percent⁹.

2.1.3 Ferric Sulfate

Ferric sulfate, a hemostatic and preserving agent, reduces the incidence of inflammation-induced internal resorption by stimulating agglutination^{9,15}. When applied to the canal orifices, the ferric and sulfate ions react with blood to achieve hemostasis. The resulting agglutination decreases the chances of an inflammatory response^{9,15}. Still, radiographic observations of internal resorption can lead to premature exfoliation and subsequent arch length loss¹⁵. However, ferric sulfate success rates (84.8 percent) are comparable to that of formocresol (87.1 percent) at 24 months¹².

2.1.4 Sodium Hypochlorite

Sodium hypochlorite has predominantly been used as an irrigant in permanent tooth root canal therapies since the 1920s^{2,9,12}. The antimicrobial properties of 3% to 5% sodium hypochlorite also make the irrigant an ideal disinfectant. In primary tooth

pulpotomies, sodium hypochlorite success rates (82.9 percent) are significantly lower than formocresol success rates (98.1 percent) at 18 months¹².

2.1.5 Calcium Hydroxide

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) was introduced in the 1930s⁹. Unlike its predecessors, $\text{Ca}(\text{OH})_2$ has a regenerative effect. The regenerative effect of $\text{Ca}(\text{OH})_2$ is due to its alkaline pH⁹. When applied to vital pulpal tissue, the high pH of $\text{Ca}(\text{OH})_2$ can stimulate reparative dentin or an inflammatory cascade^{9,16}. $\text{Ca}(\text{OH})_2$ dissociates into calcium ions and hydroxyl ions that lead to cellular differentiation and hard tissue formation^{11,16}. A superficial inflammatory reaction can initiate this repair process¹⁷. However, the solubility of $\text{Ca}(\text{OH})_2$ can also result in a continuous inflammatory reaction that can lead to pulpal necrosis and subsequent internal resorption^{4,17,18}. At 24 months, $\text{Ca}(\text{OH})_2$ success rates (41.4 percent) are significantly lower than formocresol success rates (79.0 percent)¹². Due to its low success rates and sequelae of internal root resorption, $\text{Ca}(\text{OH})_2$ is not recommended for use in primary molar pulpotomies.

2.1.6 Mineral Trioxide Aggregate

MTA was first introduced in 1993 by Torabinejad as a medicament to repair canal perforations^{9,12,19}. MTA is composed of Portland cement, bismuth oxide, dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite^{9,11,12,20}. MTA is a biocompatible material with bactericidal properties and a pH of 10.2 immediately after mixing and a pH of 12.5 three hours after mixing²¹. The mean set time of MTA is 2 hours 45 minutes \pm 5 minutes²¹. The major advantage of MTA is that it can stimulate dentinal bridging¹². Compared to $\text{Ca}(\text{OH})_2$, which also induces hard tissue

formation, MTA can achieve the same effect in a shorter period of time and with less inflammation⁴. Studies show that MTA has been reported to have high success rates. MTA and formocresol have the highest pulpotomy success rates at 24 months (89.6 percent and 85.6 percent, respectively) compared to ferric sulfate (79.3 percent)¹². MTA success rates are not significantly different than that of formocresol, but is significantly higher than that of ferric sulfate¹². MTA's long set time, discoloration effects, and difficult handling properties are major disadvantages.

2.2 Biodentine™

Biodentine™ is a calcium silicate material that stimulates secondary dentin formation^{3,4}. Biodentine™ is composed of powder and liquid components mixed to form a gel structure that polymerizes into a solid⁶. Its powder component is composed of tricalcium silicate, calcium carbonate, dicalcium silicate, calcium oxide, and iron oxide⁶. Its liquid component is composed of hydrosoluble polymer and calcium chloride⁶. When the powder and liquid components are mixed, the calcium silicate particles react with water to yield calcium, hydroxyl, and silicate ions²⁰. The resulting calcium hydroxide increases the pH to 12 while the resulting calcium silicate hydrate gels polymerize over time to form a rigid structure²⁰. The calcium silicate also interacts with phosphate ions in saliva to form apatite deposits that increase the sealing ability and decrease the microleakage of Biodentine™²⁰.

At a cellular level, Biodentine™ promotes pulpal healing by increasing cell proliferation, migration adhesion, and mRNA expression of chemokines²². Biodentine™ also induces Transforming growth factor-Beta 1 secretion that results in increased

collagen synthesis and formation of new dentin. Biodentine™ is biocompatible, non-cytotoxic, and non-genotoxic, addressing the drawbacks of formocresol²³.

Studies have revealed that there is no difference in the success rates of Biodentine™ and MTA primary molar pulpotomies^{3,5}. Compared to MTA, however, Biodentine™ has higher compressive strength, increased calcium ion release, lower porosity, better color stability, better handling, and a lower set time⁵⁻⁷. While the set time of Biodentine™ is more favorable than that of MTA, the manufacturer recommends that a Biodentine™ pulpotomy and the definitive restoration be completed in two separate visits. This remains an obstacle to its wide use in pediatric dentistry, a field in which working time relies heavily on patient cooperation.

The manufacturer recommends the placement of Biodentine™ directly on the pulp orifices up to the occlusal surface of the tooth on the first visit (Appendix B). After 12 minutes at initial set, the rubber dam and matrix may be removed. After one week to six months, a definitive restoration may be placed. A recent study has examined the placement of a definitive restoration on unset Biodentine™ using plastic teeth⁸. The authors found that Biodentine™ displacement was minimal and that it is acceptable to place the definitive restoration three minutes after the material was mixed and placed⁸. Currently, no similar evidence exists for natural teeth. This study investigates the hypothesis that Biodentine™ is a suitable material for use on a single-visit pulpotomy and definitive restoration procedure. The aim of the research is to assess the displacement and secondary set of Biodentine™ restored definitively with stainless

steel crowns in a single-visit and to compare the effect different liner materials on the displacement and secondary set of Biodentine™ in primary molar pulpotomies.

2.3 Measuring Microhardness

The setting reaction and strength of a material can be measured as a function of microhardness. Microhardness is a material's ability to resist permanent deformation when a prescribed load is applied²⁴. Microhardness is a mechanical property that is affected by other properties of the material, including surrounding pH, particle size, temperature, yield and tensile strengths, and temperature²⁴. There are two types of microhardness tests—Knoop and Vickers. These tests differ in the shape of the indenter used. The Knoop test involves the use of an elongated pyramid indenter and the Vickers test involves the use of a square pyramid diamond indenter²⁴. The load and dwell time prescribed to the indenter are determined during a pilot test in which a clear indent is visible²⁴. To increase visibility of the indent, the surface must be polished to remove the superficial layer and reveal a scratch-free area²⁴.

During indentation, the indenter applies the determined load force (F) for a determined dwell time. Following application of the load, the dimensions of the indentation are measured and the hardness is defined as the ratio of the load to the facet contact area²⁴. Knoop hardness (HK) is calculated by the following equation:

$$HK = F/A$$

F is the load (kg^{-1}) and A is the area produced by the indenter²⁴. A large HK value indicates low hardness.

3.1 Aims and Objectives

The purpose of this *in-vitro* study is to evaluate the setting reaction of Biodentine™, used as a pulpotomy agent, as a function of its hardness 24 hours after final tooth restoration. The objectives of the study are to compare the effect of different overlying materials such as Zinc Oxide Eugenol (ZOE) and Resin Modified Glass Ionomer (RMGI) cements on the setting reaction of Biodentine™ in primary molar pulpotomies restored definitively with stainless steel crowns (SSC) in a single visit. This *in vitro* trial will provide recommendations on appropriate restorative materials for Biodentine™ pulpotomies completed in a single visit.

3.2 Hypothesis

H(o): There is no difference in hardness of Biodentine™ in primary molar pulpotomies whether it is placed according to manufacturer recommendations, or restored in a single visit with SSCs, ZOE, or RMGI cements.

3 MATERIALS AND METHODS

3.1 IRB Approval

The protocol #2017-0179 was reviewed on February 22, 2017 by OPRS of IRB #7. The proposal does not involve “human subjects”, and thus an exemption was granted (Appendix A).

3.2 Overview

This *in vitro* study was conducted in the labs of Drs. Satish Alapati and Anakarina Bedran-Russo at the University of Illinois at Chicago College of Dentistry (801 S. Paulina St, Rooms 536 Chicago IL, 60612). The primary investigator (PI) prepared and tested all samples.

3.3 Dental Materials

The dental materials in this study were prepared according to manufacturer instructions.

- Biodentine™: five drops of liquid to one capsule of Biodentine™, triturated 4000 rotations/mm for 30 seconds
- Intermediate Restorative Material (IRM, Dentsply Caulk, Milford, Delaware, USA): one scoop powder to one drop liquid
- Fuji II LC (GC America Inc., Alsip, Illinois, USA): triturated for 10 seconds, lightcured for 20 seconds
- FujiCem2 Cement (GC America Inc., Alsip, Illinois, USA): applied to intaglio of SSC, excess removed

3.4 Selection and Mounting of Samples

Forty extracted primary molars were used for this study. Inclusion criteria included primary molars with caries, complete root development, and existing composite or amalgam restorations. Teeth that had gross caries compromising the integrity of the tooth, incomplete root development, or existing stainless steel crowns were excluded. The teeth were stored in 2% Chloramine-T solution. Each sample was mounted with rope wax to a plastic dappen dish. Laboratory stone was used to mount the samples.

3.5 Pilot

A pilot study was completed on 10 samples to determine the effective polishing grits and times and appropriate load force and dwell time for the Knoop hardness test.

3.6 Study Groups

Ten teeth were randomly assigned to each of four groups as follows:

Group 1: Biodentine™, ZOE (IRM), SSC with FujiCem2

Group 2: Biodentine™, RMGI (Fuji II LC), SSC with FujiCem2

Group 3: Biodentine™, SSC with FujiCem2

Group 4 (Control Group): Biodentine™ only

The study was done in two batches, with the first five samples of each group being completed during the first batch and the final five samples of each group being completed in the second batch.

3.7 Preparation of Samples

The clinical pulpotomy procedure was replicated in each sample. Occlusal cavities were prepared to the furcation with a round bur in a high speed handpiece and pulpal debris was

removed with a round bur in a slow speed handpiece and a spoon excavator. The pulp chambers were irrigated with 0.12% Chlorhexidine and dried with air and cotton pellets. The pulpal depth was recorded from the pulpal floor at the furcation to the cavosurface margin. Following cavity preparation and tissue excavation, the samples were restored according to assigned group as illustrated in Figure 1.

Group 1: Biodentine™, IRM, SSC

Biodentine™ was placed over the radicular orifices and pulpal floor to an approximate thickness of three mm and allowed to set for three minutes. IRM was placed in the remaining cavity and the tooth was then prepared to receive an SSC. An SSC was then fitted and cemented with FujiCem2.

Group 2: Biodentine™, RMGI, SSC

Biodentine™ was placed over the radicular orifices and pulpal floor to an approximate thickness of three mm and allowed to set for three minutes. Fuji II LC was placed in the remaining cavity and the tooth was then prepared to receive an SSC. An SSC was then fitted and cemented with FujiCem2.

Group 3: Biodentine™, SSC

Biodentine™ was placed over the radicular orifices and pulpal floor to the occlusal surface. The tooth was then prepared to receive an SSC. An SSC was then fitted and cemented with FujiCem2.

Group 4 (Control): Biodentine™ only

Biodentine™ was placed over the radicular orifices and pulpal floor to the occlusal surface with no definitive restoration.

3.8 Storage of Samples

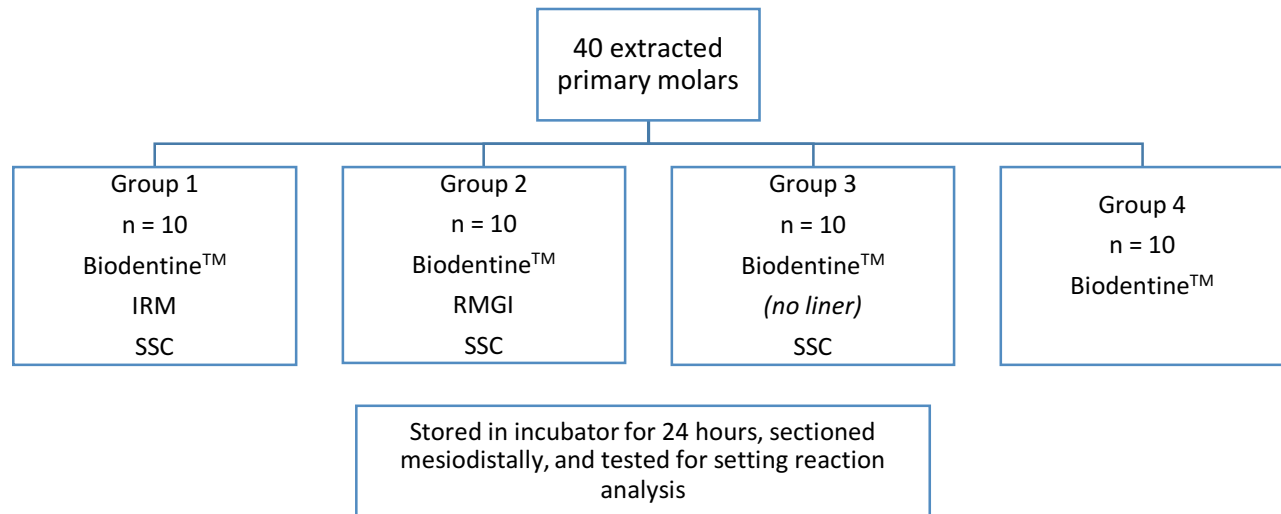
Following preparation, the samples were stored in pipette boxes at 100% humidity which were stored in an incubator at 37°C (98.7°F) for 24 hours to simulate the environmental conditions of the oral cavity.

3.9 Sectioning, Polishing, and Testing Samples

After 24 hours, the samples were sectioned mesiodistally with a diamond cut-off wheel, polished using a EcoMet 3000 Variable Speed Grinder-Polisher (Buehler, Lake Bluff, Illinois, USA) at 600 grit, 800 grit, and 1200 grit, and mounted to a slide with sticky wax.

Each sample was tested for hardness with a Leco Microhardness Tester LM700AT (LECO, Saint Joseph, Michigan, USA). Each sample was visualized under the light microscope at 10x magnification and the Biodentine™ material interface was identified. Each sample was measured for microhardness with 50gf load force for 30 seconds in three zones: 1mm, 2mm, and 3mm from the Biodentine™ material interface. The hardness was recorded in Knoop Scales (HK).

Figure 1: Study Design Flow Chart



3.10 Statistical Analysis

GraphPad Prism software (GraphPad Software, La Jolla California USA) was used to analyze the collected data. Analysis of Variance (ANOVA) with post-hoc Tukey multiple comparisons testing was used to compare the three zones within each group and each zone across groups.

4 RESULTS

This *in vitro* study included the preparation of 40 extracted teeth, with 10 teeth in each of the four groups. The hardness values (HK) were measured at three zones in each sample: 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3). HK at each zone was averaged in the four groups. Table 1, Table 2, and Table 3 report the hardness data and mean HK at Zones 1, 2, and 3 in Groups 1, 2, and 3 respectively.

4.1 Comparison of Zones Within Groups

Figure 2, 3, 4, and 5 illustrate the distribution of HK measurements in Zones 1, 2, and 3 for each group. The solid bar indicates the mean hardness and the line indicates the standard deviation. One-way ANOVA comparison of the three zones within each group showed no significant difference in hardness overall ($P>0.05$). Tukey's post-hoc comparison showed no significant differences in hardness between Zones 1 and 2, Zones 1 and 3, and Zones 2 and 3 of any of the groups.

4.2 Comparison of Zones Across Groups

ANOVA with Tukey's post-hoc was also completed to compare zones across all groups. Figure 6, 7, and 8 show the distribution of hardness values, mean value, and standard deviation in Zones 1, 2, and 3 for each group. There was no significant difference in hardness overall nor among groups in any of the zones ($P>0.05$). Figures 9, 10, and 11 display the confidence intervals for the difference between the means of hardness. The vertical line at zero represents the grand mean, or normalized mean. The grand mean falls within all the confidence intervals, which indicated that the difference between these means was not statistically significant. The

95% simultaneous confidence level indicated that the study was 95% confident that all these confidence intervals contained the true differences.

Table 1: Group 1 (BD-IRM-SSC) Hardness Values

Sample #	Zone 1	Zone 2	Zone 3
1	68.1	57.1	49.7
2	55.5	57.6	63.8
3	75.3	60.1	54.4
4	47.2	59.1	64
5	64.5	64.5	61.6
6	40.8	51.7	47.6
7	57.8	60.9	56.1
8	62.6	54.9	44
9	45.9	59.7	56.5
10	48.2	43.8	45.1
Mean	56.59	56.94	54.28
SD	11.08577166	5.772001386	7.47214085

Hardness values measured in Knoop Hardness (HK) at three zones in each sample: 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3); BD = Biodentine; IRM = Interim Restorative Material; SSC = Stainless Steel Crown; SD = standard deviation

Table 2: Group 2 (BD-RMGI-SSC) Hardness Values

Sample #	Zone 1	Zone 2	Zone 3
11	60.2	54.4	57.7
12	60.1	44.8	37.3
13	60.6	60.3	65.7
14	50.8	55.6	51.3
15	26.4	43.5	52.5
16	45.4	48.7	53.4
17	24.8	45.5	57.4
18	30.1	41.9	59.1
19	57.2	41.3	43.7
20	50.4	32.5	34.4
Mean	46.6	46.85	51.25
SD	14.39374864	8.140195193	9.953028573

Hardness values measured in Knoop Hardness (HK) at three zones in each sample: 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3); BD = Biodentine; RMGI = Resin Modified Glass Ionomer; SSC = Stainless Steel Crown; SD = standard deviation

Table 3: Group 3 (BD-SSC) Hardness Values

Sample #	Zone 1	Zone 2	Zone 3
21	57.9	58.9	56.8
22	75.6	73.5	68.8
23	80.7	62	92.5
24	90.6	86.1	93.1
25	81.4	55.1	83.8
26	35.5	35.4	48.6
27	46.1	38.7	32.8
28	48	41.2	54.9
29	44	50.6	52.5
30	59.6	60.6	49.3
Mean	61.94	56.21	63.31
SD	18.93428401	15.81156047	20.44768121

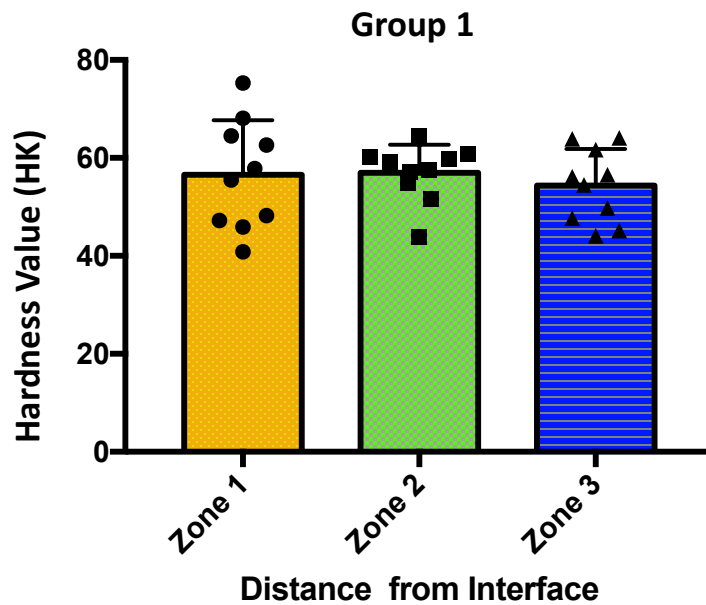
Hardness values measured in Knoop Hardness (HK) at three zones in each sample: 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3); BD = Biodentine; SSC = Stainless Steel Crown; SD = standard deviation

Table 4: Group 4 (BD) Hardness Values

Sample #	Zone 1	Zone 2	Zone 3
31	50.6	56.4	47.6
32	47.2	46.9	57.1
33	55.2	50.2	61
34	65.3	63	67.3
35	68.9	62.4	60.7
36	55.5	44.5	43.7
37	57.1	62.5	69.9
38	46.3	51.5	48.6
39	64.8	67.1	65.9
40	42.3	42.4	47.6
Mean	55.32	54.69	56.94
SD	8.920612834	8.770715162	9.460702346

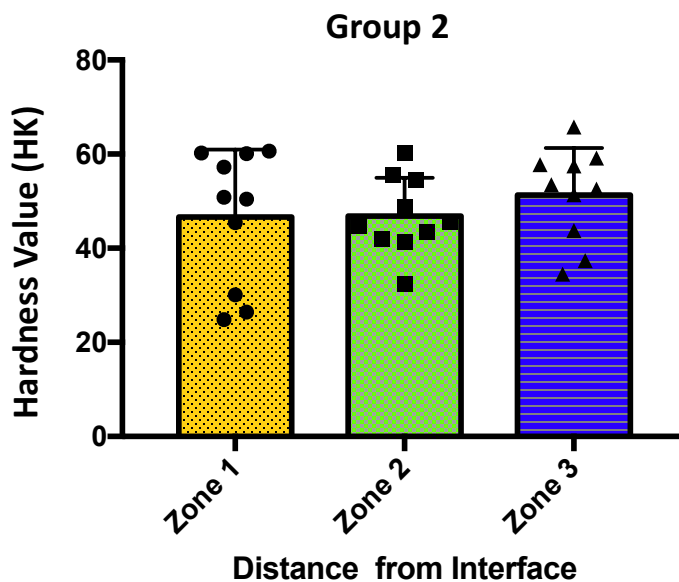
Hardness values measured in Knoop Hardness (HK) at three zones in each sample: 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3); BD = Biodentine; SD = standard deviation

Figure 2: Comparison of Hardness Value Distribution in Group 1



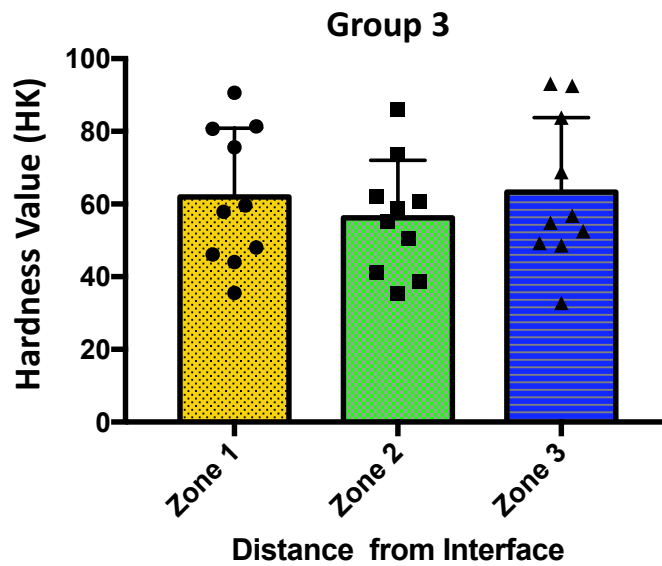
Hardness values measured in Knoop Hardness (HK) at three zones in Group 1 (Biodentine, Interim Restorative Material, and Stainless Steel Crown): 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3)

Figure 3: Comparison of Hardness Value Distribution in Group 2



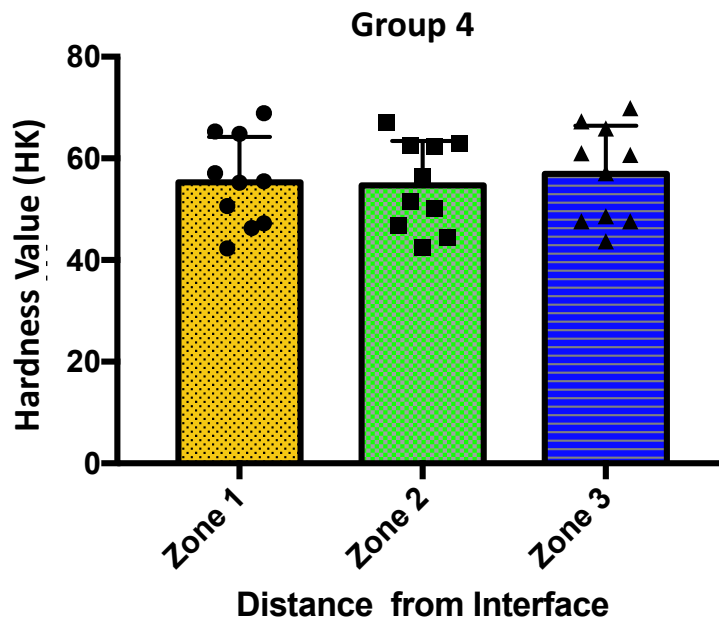
Hardness values measured in Knoop Hardness (HK) at three zones in Group 2 (Biodentine, Resin Modified Glass Ionomer, and Stainless Steel Crown): 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3)

Figure 4: Comparison of Hardness Value Distribution in Group 3



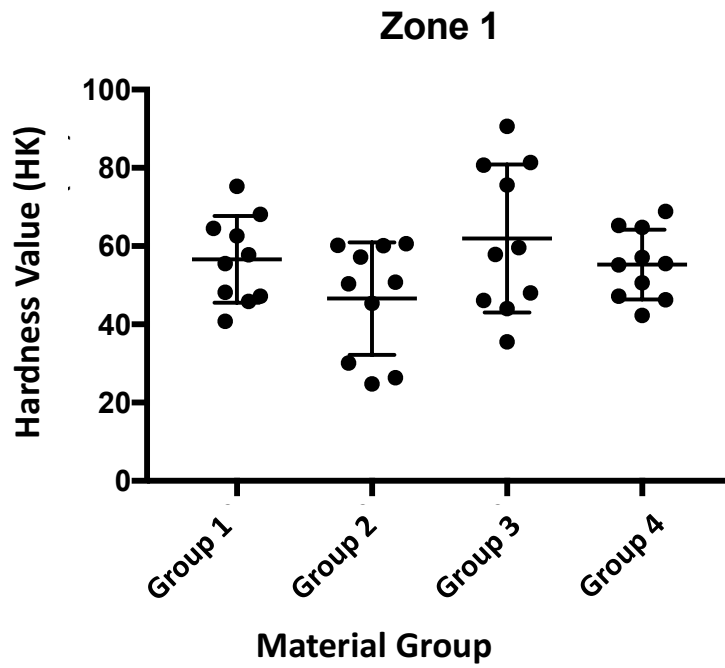
Hardness values measured in Knoop Hardness (HK) at three zones in Group 3 (Biodentine and Stainless Steel Crown): 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3)

Figure 5: Comparison of Hardness Value Distribution in Group 4



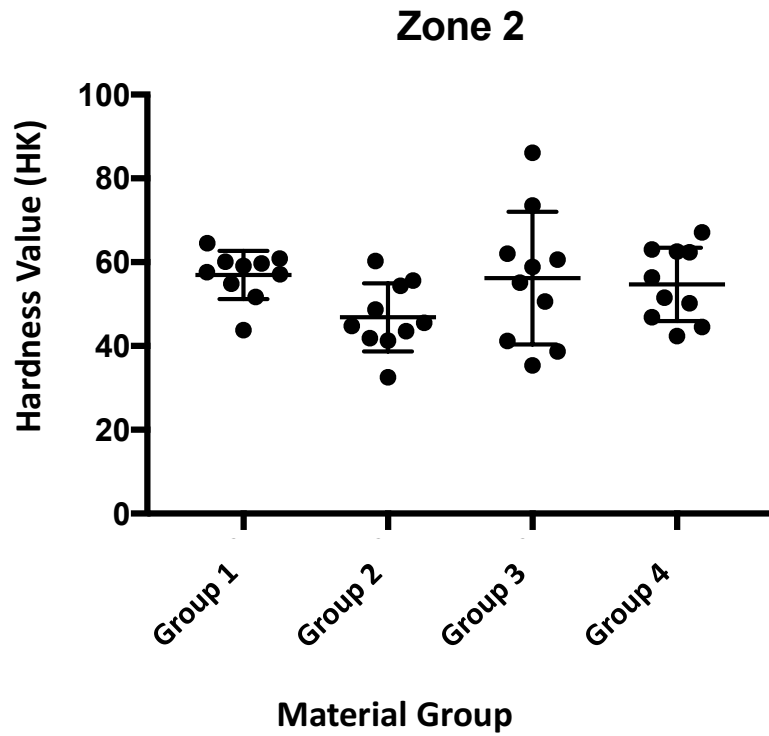
Hardness values measured in Knoop Hardness (HK) at three zones in Group 4 (Biodentine): 1mm (Zone 1), 2mm (Zone 2), and 3mm (Zone 3)

Figure 6: Comparison of Hardness Value Distribution in Zones 1



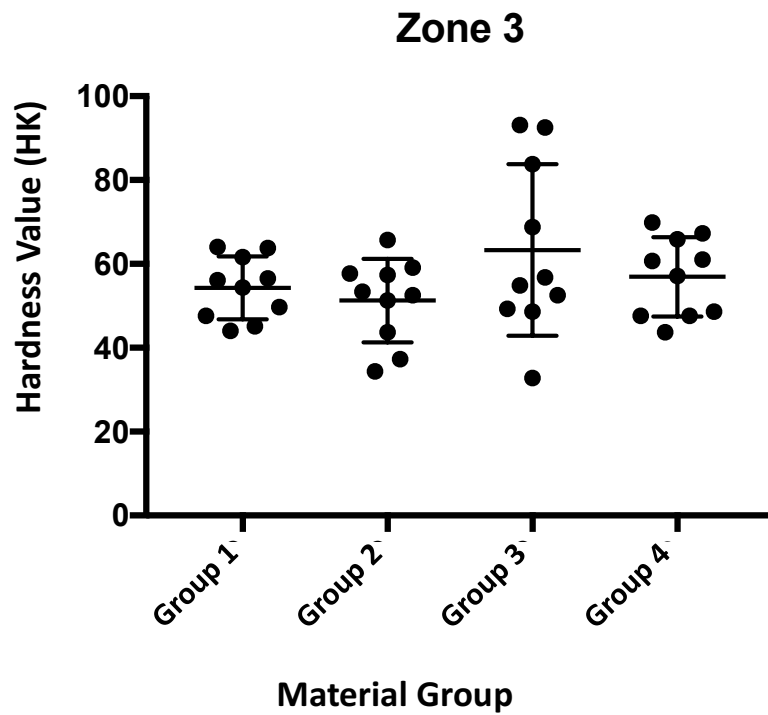
Group 1 = Biodentine, Interim Restorative Material, SSC; Group 2 = Biodentine, Resin Modified Glass Ionomer, SSC; Group 3 = Biodentine, SSC; Group 4: Biodentine

Figure 7: Comparison of Hardness Value Distribution in Zones 2



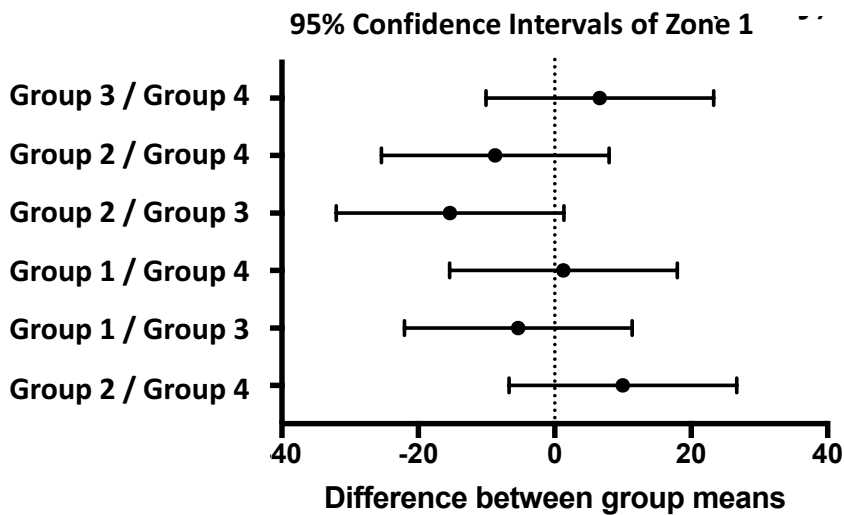
Group 1 = Biodentine, Interim Restorative Material, SSC; Group 2 = Biodentine, Resin Modified Glass Ionomer, SSC; Group 3 = Biodentine, SSC; Group 4: Biodentine

Figure 8: Comparison of Hardness Value Distribution in Zones 3



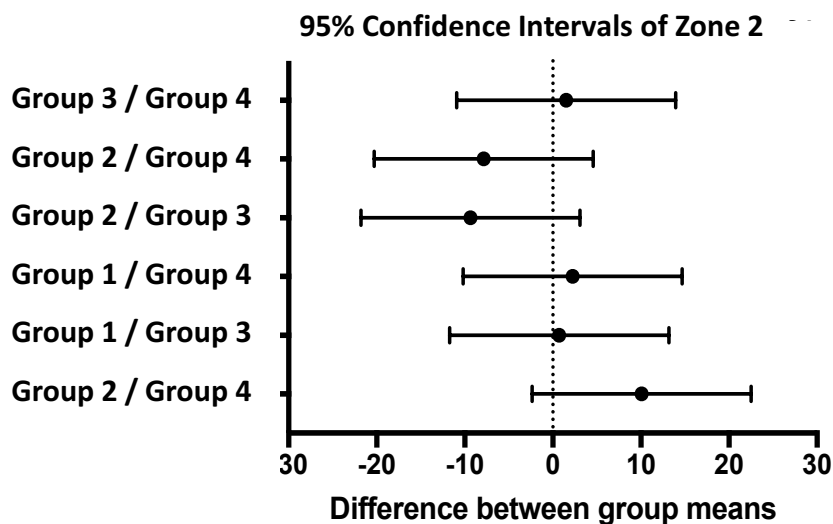
Group 1 = Biodentine, Interim Restorative Material, SSC; Group 2 = Biodentine, Resin Modified Glass Ionomer, SSC; Group 3 = Biodentine, SSC; Group 4: Biodentine

Figure 9: Zone 1 Confidence Intervals



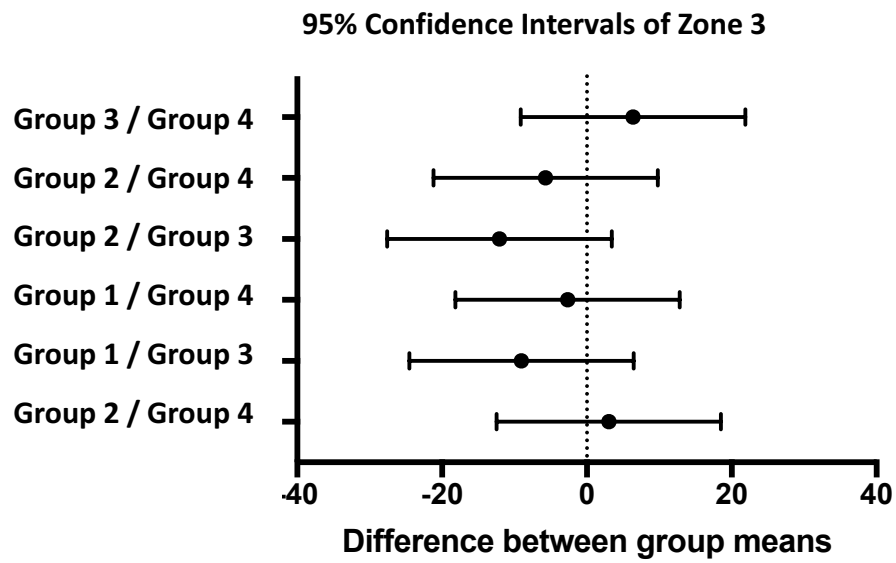
Group 1 = Biodentine, Interim Restorative Material, SSC; Group 2 = Biodentine, Resin Modified Glass Ionomer, SSC; Group 3 = Biodentine, SSC; Group 4: Biodentine

Figure 10: Zone 2 Confidence Intervals



Group 1 = Biodentine, Interim Restorative Material, SSC; Group 2 = Biodentine, Resin Modified Glass Ionomer, SSC; Group 3 = Biodentine, SSC; Group 4: Biodentine

Figure 11: Zone 3 Confidence Intervals



Group 1 = Biodentine, Interim Restorative Material, SSC; Group 2 = Biodentine, Resin Modified Glass Ionomer, SSC; Group 3 = Biodentine, SSC; Group 4: Biodentine

5 DISCUSSION

For young children who may be unwilling or unable to cooperate for extended periods of time and across multiple appointments, the duration and frequency of dental visits is a concern. This *in vitro* study examined the necessity of a postponing final restoration by comparing the effect of ZOE and RMGI as liners and the effect of a definitive SSC restoration on the setting reaction of Biodentine™ in primary molar pulpotomies. This study supports recent findings that a definitive SSC restoration can be seated on Biodentine, used as a pulpotomy agent, three minutes after mixing.⁸ There have been no similar studies evaluating the setting reaction of Biodentine in primary molar pulpotomies restored in a single visit involving natural teeth. The results in this *in vitro* study indicate that there is no difference in hardness of Biodentine in primary molars restored in a single visit with or without either ZOE or RMGIC liners.

The manufacturer recommendation for Biodentine use in pulpotomy procedures is to place the Biodentine on the pulpal orifices and floor to the occlusal surface, to serve as both a pulpotomy agent and a temporary restoration.²⁵ In this study, Biodentine was placed to a thickness of three mm in Groups 1 and 2, and to the occlusal surface in Groups 2 and 3. A comparison of Biodentine thickness showed no significant difference in hardness. Therefore, contrary to the manufacturer recommendations, three mm is an adequate material thickness. Clinically translated, less Biodentine material can be used, introducing a more cost-effective protocol for the practitioner.

Setting reaction was measured as a function of microhardness for this study. The Knoop test measures for indentation created by a predetermined load force and dwell time.^{23,24} The Knoop test requires only a small sample and causes minor damage to the sample, however,

error may arise from the polishing pressure that may cause microdefects resulting in increased microhardness values.²⁴ However, each sample was polished with the same protocol (600 grit, 800 grit, and 1200 grit for 10 minutes each), which would make the microdefects uniform across all samples.

Other measures of setting reaction for future studies may include the Gillmore needle test, compressive strength studies, and analysis of porosity. The Gillmore needle tests for setting time of a cement material by repeatedly applying a weighted needle onto the material surface and measuring the elapsed time from the mixing to when an attempted indentation is unsuccessful.^{21,24} The Gillmore test was not appropriate for this study, as the liners and restorations were placed three minutes after mixing.

While preparing the samples to receive SSCs, Biodentine washout was observed, consistent with a recent study that reported Biodentine demonstrated greater washout compared to Bioaggregate or Intermediate Restorative Material (**IRM**).²³ The low washout rates of IRM is attributed to the eugenol liquid that is not water miscible.²³ IRM was used as the ZOE liner in this study. This would suggest that IRM as a liner would be effective to prevent Biodentine washout when preparing the tooth to receive a SSC. In addition to washout, subjecting the unset Biodentine to water could increase the water to cement ratio and introduce voids. Measuring voids proves to be challenging because pores are multi-dimensional and difficult to identify and classify in a cross-section surface view.²⁴ Biodentine, like other calcium silicate cements, require moisture to set.

This study design aimed to replicate the pulpotomy and restorative techniques used in the clinical setting. However, the availability of resources created limitations to fully simulate

the clinical setting and complexity of the oral environment, such as blood and saliva contamination, changing thermal and pH conditions, and chewing forces that may affect dental material setting reactions. The variability of these conditions affect the material's reported microhardness.²⁴ Microhardness is a mechanical property that is affected by other properties of the material, including surrounding pH, yield and tensile strengths, and temperature.²⁴

While oral conditions were simulated with samples stored in 100% humidity at 37°C in this study, access to a saliva medium or thermal cycling chambers would more accurately replicate natural oral conditions in which eating would expose the human mouth to a range of temperatures and salivary gland stimulation. Furthermore, altering the surrounding pH and applying mechanical occlusal forces to the samples would enhance the likeness to natural intraoral conditions. Introduction of a saliva medium would provide phosphate ions that interact with calcium silicate to form apatite deposits; this formation increases the sealing ability and decreases the microleakage of Biodentine.^{20,26} Therefore, no difference in findings is expected with a saliva medium. Further investigation is necessary to determine the clinical success of this study's findings. Future studies may investigate the clinical and radiographic success rates of primary molars that have pulpotomies and definitive restorations completed on the same visit with or without liners.

Biodentine samples were donated by Septodont for this study. The authors did not receive any additional funding and report no conflicts of interest.

6 CONCLUSION

The following conclusions can be made based on the results of this study:

- 1) IRM, RMGI, and SSCs as overlying liners and restorative materials have no effect on the quality of setting reaction of Biodentine™.
- 2) Biodentine™ pulpotomies can be restored definitively during the same visit with no effect on setting reaction

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APPENDIX A

2017-0179 Page 1 of 2 February 22, 2017

Determination Notice Research Activity Does Not Involve "Human Subjects"

February 22, 2017

Chi-Lan Pham
Pediatric Dentistry
801 S. Paulina Street
M/C 850
Chicago, IL 60612
Phone: (312) 996-7532 / Fax: (312) 413-8006

**RE: Research Protocol # 2017-0179
"In Vitro Evaluation of Biodentine Material Displacement in Primary Molar
Pulpotomy Procedure"**

Sponsor(s): None

Dear Chi-Lam Pham:

The above proposal was reviewed on February 22, 2017 by OPRS staff/members of IRB #7. From the information you have provided, the proposal does not appear to involve "human subjects" as defined in 45 CFR 46.102(f).

The specific definition of human subject under 45 CFR 46.102(f) is:

Human subject means a living individual about whom an investigator (whether professional or student) conducting research obtains

- (1) data through intervention or interaction with the individual, or
- (2) identifiable private information.

Intervention includes both physical procedures by which data are gathered (for example, venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes. *Interaction* includes communication or interpersonal contact between investigator and subject. *Private information* includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record). Private information must be individually identifiable (i.e., the identity of the subject is or may readily be ascertained by the investigator or associated with the information) in order for obtaining the information to constitute research involving human subjects.

All the documents associated with this proposal will be kept on file in the OPRS and a copy of this letter is being provided to your Department Head for the department's research files.

If you have any questions or need further help, please contact the OPRS office at (312) 996-1711 or me at (312) 355-2908.

Sincerely,
Charles W. Hoehne, B.S.

Assistant Director, IRB # 7
Office for the Protection of Research Subjects

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
Ian Marion, Pediatric Dentistry, M/C 850

VITA

CHI-LAN T. PHAM, DDS

EDUCATION

University of Illinois at Chicago College of Dentistry
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Residency: Pediatric Dentistry
Expected June 2018: Certificate and M.S. Oral Sciences

July 2016 – Present

Howard University College of Dentistry
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Doctor of Dental Surgery

August 2012 – May 2016

University of Virginia
Charlottesville, VA
Bachelor of Science, Biology

August 2007 – May 2011

THESIS & RESEARCH

Effect of Overlying Material on Biodentine Setting Reaction in Primary Molar Pulpotomies
Thesis Defense: April 3, 2018

LICENSURE & CERTIFICATION

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March 2016
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Current
Current
Scheduled: May 7, 2018

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President

March 2014 – March 2016

Students United for America's Toothfairy
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Secretary

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Committee Member & Volunteer

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SNDA Oral Cancer Walk, Volunteer
Jamaica Dental Mission, Volunteer
March Mouth Gladness!, Volunteer
Girl Scouts Healthy Living Fair, Volunteer
Vietnamese Medical Society of the Northeast America

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March 2014 – 2016
July 2015
March 2015
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C2 Education
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2014, 2015
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2015 – Present
2016 – Present
2016 – Present

SPECIAL SKILLS

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