CHAPTER 3

Materials and Methods

3.1 Research design

Superiority randomized controlled trial

3.2 Materials

- X-ray film No.2 (Insight®; Kodak; Rochester, NewYork, USA)
- Digital imaging plate size 2 (CS 7606, Carestream Dental, Noisy Le Grand, Paris, France)
- Film holder (Snap-A-Ray® and XCP®; Dentsply; Elgin, Illinois, USA)
- Digital imaging software 6.3.0.0 (Trophy DICOM, Marne la Valèe, France)
- Endo Ice (Green Endo-Ice®; ColteneWhaledent; Cuyahoga Falls, Ohio, USA)
- Topical anesthesia (One touch®; Hager Worldwide; Ontario, Canada)
- Short needle 21 mm. gauge No.27 (Terumo Dental Needle; Terumo Corporation; Tokyo, Japan)
- 4% articaine with 1: 100,000 epinephrine (Septanest SP; Septodont; Saint-Maur-des Fasses Cedex, France)
- Rubber dam sheet
- Rubber dam clamp Ivory No.14 and 14A
- High-speed football shaped diamond bur (Jota AG, Montagnola, Switzerland)
- High-speed thin taper diamond bur (Jota AG, Montagnola, Switzerland)
- High-speed round diamond bur diameter 1and 1.5 mm. (Jota AG, Montagnola, Switzerland)
- Low-speed round steel burs diameter 1and 1.5 mm. (Jota AG, Montagnola, Switzerland)
- Spoon excavator
- 2 % Chlorhexidine gluconate solution (Faculty of Dentistry Chiang Mai University, Chiang Mai, Thailand

- 37% phosphoric acid (*Scotchbond*™; 3M;St. Paul, Minnesota, USA)
- Adhesive (Adper™ Single Bond; 3M;St. Paul, Minnesota, USA)
- Calcium hydroxide liner material (Dycal®; Dentsply; Elgin, Illinois, USA)
- Resin-modified glass ionomer base material (Vitrebond™; 3M; St. Paul, Minnesota, USA)
- Biodentine™ (Septodont; Saint-Maur-des-FassesCedex, France)
- Resin composite (Filtek™ Z350 XT; 3M; St. Paul, Minnesota, USA)
- Stainless steel crown (3M; St. Paul, Minnesota, USA)
- Self-cured resin modified glass ionomer cement (RelyX™ Luting 2; 3M; St. PaulmMinnesota, USA)

3.3 Research populations and samples

This study was approved by the Research Ethics Committee of the Faculty of Dentistry, Chiang Mai University.

3.3.1 Study populations

All subjects participating in this study were recruited from patients age 6-18 years old attending the Pediatric Dentistry Clinic, Faculty of Dentistry, Chiang Mai University. All subjects had permanent teeth with deep carious lesions, diagnosed from clinical and radiographic examinations. Teeth were diagnosed as normal pulp or reversible pulpitis.

3.3.2 Patient selection and examination

Following a clinical examination, a bitewing radiograph of each deep carious lesion was obtained to evaluate the depth of lesions. A parallel periapical radiograph was obtained to evaluate periapical tissue. All radiographs were taken by using X-ray film No.2 (Insight®) or digital imaging plate size 2 (CS 7606). The subject who had at least one permanent tooth that met the inclusion criteria was invited to participate in the study. The study information then was explained to the legal guardians and the patients, and both were asked to decide whether to participate in the study. If they agree to participate, the legal guardians and the patients were asked to sign informed consent and the assent form, respectively.

3.3.3 Sample groups

All permanent teeth with deep carious lesions were screened clinically according to inclusion and exclusion criteria as follow:

Patients criteria

1. Patients aged between 6-18 years old who had one permanent tooth with deep carious lesions.

2. The legal guardians and the patients received adequate information and signed informed consent and the assent form.

Clinical criteria

A. Clinical inclusion criteria

1. Permanent tooth with deep carious lesions

2. The recruited tooth had positive response to cold test (Green Endo-Ice®), resulting in the diagnosis of normal pulp or reversible pulpitis

3. Absence of clinical swelling

4. Absence of pus exudates/ fistula of soft and periodontal tissues

5. Absence of abnormal tooth mobility and pain on percussion

6. Restorable with resin composite or stainless steel crown (SSC)

7. After complete caries removal that make it to see the pulp by transparency

B. Clinical exclusion criteria

1. Teeth did not see the pulp by transparency after complete caries removal.

2. A pulp exposure occurred during caries removal.

3. Excessive tooth destruction that cannot be restored with resin composite or SSC.

Radiographic criteria

A. Radiographic inclusion criteria

From a posterior bitewing radiograph

1. The extension of the dental caries radiolucency penetrates into three fourths or more of the entire dentin thickness.

From a periapical radiograph

1. Absence of radiolucencies at periapical regions

2. Absence of internal and/or pathologic external root resorption
3. Absence of calcification or pulp canal obliteration

3.3.4 Sample size calculation

Calculation based on the formula:

$$n = f(\alpha/2, \beta) \times [p_1 \times (100 - p_1) + p_2 \times (100 - p_2)] / (p_2 - p_1)^2 \quad (101)$$

Where p_1 and p_2 are the percent 'success' in the control and experimental group, respectively**.**

Based on the study of Leye Benoist et al. (72), success rate was 73% in the CH and GI group and 93% in the MTA group, respectively. To date, there have been no studies reporting the success rate of Biodentine™. The expected success rate of Biodentine™ in this study was 98% which, higher than that of MTA. To conduct a superiority-trial with significant difference (80% power, one-sided 5% significance level), a total of 56, and 28 samples per group was needed. Seven percent of the required sample size was added to compensate for follow-up loss. Therefore, a sample size of 30 samples for each group would be appropriate for this study.

3.3.5 Random sampling

When the subjects met all the inclusion criteria, the tooth was randomly assigned into two groups of pulp protection materials, using simple block randomization with block of four. The tooth was randomly allocated into

Group 1: Dycal® and Vitrebond™ (DV)
Group 2: Biodentine™ (B)

3.4 Treatment protocol

1. Demographic data including age, gender, stage of root development and diagnosis were recorded.

2. All teeth were treated by four unblinded postgraduate students in pediatric dentistry under the same protocol and supervision of one instructor.

3. Cold test (Green Endo-Ice®) was used to test the sensibility leading to diagnosis of the recruited teeth. Green Endo-Ice® was sprayed on a cotton pellet held with cotton plier. The cold pellet was placed on buccal surface of the tooth. If buccal

surface was destructed, the cold pellet was placed on occlusal surface of the tooth. Patient may respond as follows:

- No response to Green Endo-Ice® mean that the tooth had non-vital pulp.
- Positive response to Green Endo-Ice® but the response disappeared immediately after removal of Green Endo-Ice® mean that the tooth had normal pulp.
- Sharp/immediate response to Green Endo-Ice® which subsided quickly after removal Green Endo-Ice® mean that the tooth had reversible pulpitis.
- Sharp/immediate response to Green Endo-Ice® which had prolonged duration after removal of Green Endo-Ice® mean that the tooth had irreversible pulpitis.

Only teeth diagnosed with normal pulp and reversible pulpitis were included into the study.

4. Topical anesthesia (One touch®) was applied. Then, a local anesthesia 4% articaine with 1:100,000 epinephrine (Septanest SP) was administered and rubber dam isolation was placed. (Figure 3.1)

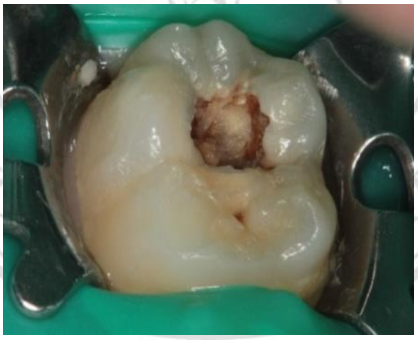

Figure 3.1 Rubber dam isolation was placed after administration of a local anesthesia.

5. A high-speed round diamond bur with diameter of 1 or 1.5 mm was used to open the access. Then, a low speed round steel bur with diameter of 1 or 1.5 mm and a spoon excavator were used to remove caries. Caries was removed from the lateral wall to the nearest point to the pulp. Removal of dental caries was continued with a spoon excavator until the hardness of dentin was felt that make it to see the pulp by transparency. If the tooth did not see the pulp by transparency or pulp exposure occurred,the tooth was excluded from the study. (Figure 3.2- 3.3)

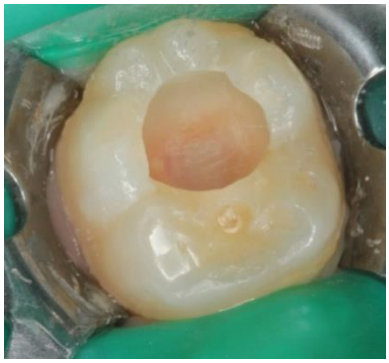

Figure 3.2 Caries was removed until make it to see the pulp by transparency.

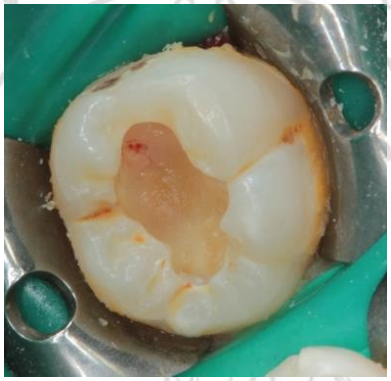

Figure 3.3 Pulp exposure occurred and the tooth was excluded.

6. The cavity was then irrigated with 5 ml of 2% chlorhexidine and dried with cotton pellet. Then, the tooth was randomly assigned by simple block randomization into 2 groups of pulp protection materials. The materials was mixed and applied according to manufacturer's instruction.

- Group 1: Dycal® and Vitrebond™ (DV group)

Dycal® contains 2 pastes of base and catalyst;Dycal® was applied in the mixing pad and mixed immediately using a dycal applicator until a uniform color was achieved. It was placed into the cavity with a dycal applicator at the point of the pulp transparency. The maximum setting time was 10 seconds. Then, Dycal® was covered with RMGI base material (Vitrebond^{TM}) which derived from mixing one scoop of powder and one drop of liquid (a powder-to-liquid-ratio of 1.4/1.0 by weight) together within 10-15 seconds. It was placed into the cavity 2 mm. thickness with a plastic instrument and a plugger, leaving the remaining space of 2-3 mm. for resin composite

restoration. For restoration with stainless steel crown, Vitrebond™ was filled into thewhole cavity. Then, the light cure was applied for 30 seconds. (Figure 3.4)

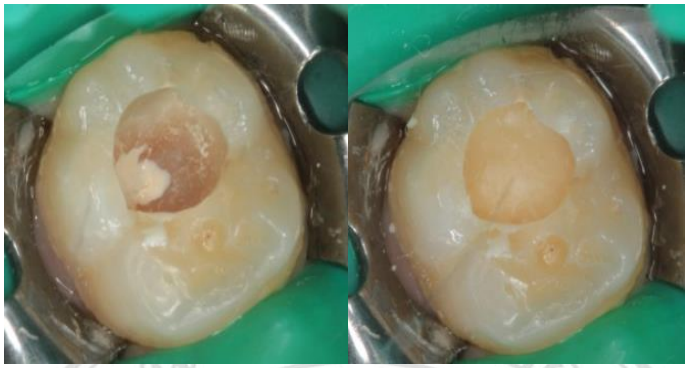

Figure 3.4 Dycal® was applied and then was covered with Vitrebond™

- Group 2: Biodentine™ (B group)

Biodentine™ contains a capsule of powder and a single-dose of liquid. After a capsule was opened and 5 drops of liquid was poured into the capsule, the capsule was closed and placed on a mixing device (Amalgamator speed of 4000 - 4200 rotations/min) for 30 seconds. Then, Biodentine™ was collected with a plastic spatula supplied in the box and applied to the cavity floor 2 mm. thickness with a plastic instrument and a plugger leaving the remaining space of 2-3 mm. for resin composite restoration. For restoration with stainless steel crown, Biodentine™ was applied as a bulk-filled into the cavity and flatted the material without excessive pressure and ensured good adaptation to the cavity walls and margins. Then, Biodentine™ was allowed to set for 12 minutes and the permanent restoration was placed. (Figure 3.5)

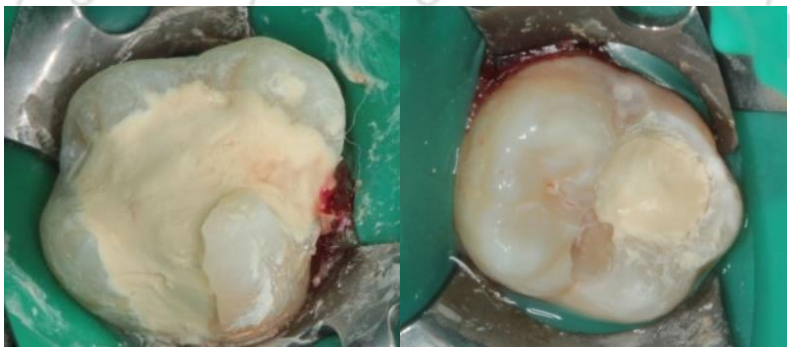

Figure 3.5 Biodentine™ was applied into the cavity.

7. For the tooth planned for resin composite restoration, the tooth was etched with 37% phosphoric acid (*Scotchbond™).* Bonding (Adper™ Single Bond) was applied and restored with resin composite (Filtek™ Z350 XT). Resin composite was applied using incremental technique (maximum: 2 mm thickness layer). Each increment was light-cured for 20 s. (Figure 3.6)

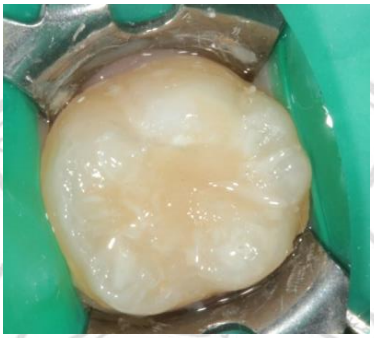

Figure 3.6 the tooth was restored with resin composite.

For the tooth planned for SSC, after tooth preparation, SSC (3M) was try in and then cemented with self-cured resin modified glass ionomer cement (RelyX™ Luting 2). (Figure 3.7)

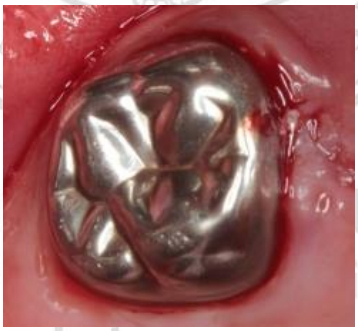

Figure 3.7 the tooth was restored with SSC.

8. Parallel and bitewing radiographs were taken immediately after the final restoration to serve as baselines.

3.4.1 Follow up period

Clinical examination

Approximately after 6 months, patients were called back for follow ups. Two unblinded examiners performed clinical examinations according to the same clinical criteria under supervision of one instructor. After clinical examinations, if there was a dislodgement of restoration that involved the base layer, the tooth was excluded from the study. To be considered as clinical favorable outcomes, the tooth has to meet all of favorable criteria. If the tooth met one clinical unfavorable criteria, the tooth was recorded as clinical unfavorable outcomes. The tooth was evaluated according to criteria as follow:

Clinical favorable criteria

- No history of pain
- Positive to cold test (Green Endo-Ice®) leading to diagnosis of normal pulp.
- Absence of fistula or swelling
- Absence of abnormal tooth mobility

Clinical unfavorable criteria

- Persistence of postoperative sensitivity after its possible causes have been managed
- History/presence of spontaneous pain
- Cold test (Green Endo-Ice®) leading to diagnosis of pulp necrosis, reversible pulpitis or irreversible pulpitis
- Presence of fistula or swelling
- Presence of abnormal tooth mobility

Discoloration was compared between immediate postoperative and follow- up photographs but was not considered as an unfavorable or favorable criterion.

Radiographic examination

Parallel and bitewing radiographs were taken after clinical examination. For radiographic examination, two blinded examiners were calibrated with the standard examiner and the agreements were 90% and 100%. Then, two examiners independently evaluated the set of radiographs twice, one week apart. Intra-examiner reliabilities were

90% and 100%. Inter-examiner reliability was 95%. Follow-up radiographs were compared with the immediate postoperative radiographs. If there was a presence of secondary caries, the tooth was excluded from the study. To be considered as radiographic favorable outcomes, the tooth has to meet all of favorable criteria. If the tooth met one radiographic unfavorable criterion, the tooth was recorded as radiographic unfavorable outcomes. The tooth was evaluated according to criteria as follow:

Radiographic favorable criteria

- Immature tooth has continued its root formation
- Intact lamina dura

Radiographic unfavorable criteria

- Presence of radiolucencies at periapical lesions
- Presence of internal and/or external root resorption

Pulp calcification and dentin bridge formation were also evaluated in bitewing radiographs but were not considered as unfavorable or favorable outcomes.

Any tooth that present clinical or radiographic signs or symptoms of irreversible pulpitis or necrosis was recorded as unfavorable outcomes and referred for vital pulp treatment or root canal treatment.

To be considered to have overall favorable outcomes, the tooth evaluated must has both clinical and radiographic favorable outcomes.

3.5 Data analysis

All data were analyzed with SPSS 19.0 software (SPSS Science, Chicago, Illinois, USA). The baseline variables between two groups were compared by the Chi-square test, t-test and Fisher's exact test. The outcomes between two groups were compared by the Fisher's Exact Test at 95% confidence interval, which considered significant difference if $p < 0.05$.

3.6 Location of study

Pediatric Dentistry Clinic, Department of Orthodontics and PediatricDentistry, Faculty of Dentistry, Chiang Mai University