CHAPTER 3

RESEARCH METHODOLOGY

Research Questions

A. Primary Question

Does a new local pit and fissure sealant, Chula Dent, show the same retention rate as the standard imported material, allowing for 10% difference, when placed on first permanent molars?

B. Secondary Question

Is there any difference in the pattern of sealant loss between the two groups concerning type, location of sealant loss, and caries development?

Research Objectives

A. Primary Objective

To compare retention rates of a recently developed local sealant (Chula Dent) with a standard imported sealant (Concise™) on first permanent molars at the period of 6 months.

B. Secondary Objectives

1. To determine the type(s) and location(s) of sealant loss of both materials.
2. To assess caries development of the teeth receiving both treatments.

Research Hypothesis

A. Research hypothesis

A new local sealant (Chula Dent) does not differ more than 10% in retention from the conventional imported sealant (Concise™) when placed on first permanent molars for the period of 6 months.

B. Statistical hypothesis

Null hypothesis $|R_1 - R_2| \geq \Delta$

Alternative hypothesis $-\Delta < R_1 - R_2 < \Delta$

R_1 : retention of the conventional sealant (Concise™)

R_2 : retention of an experimental sealant (Chula Dent)

Δ : acceptable boundary for clinical equivalence

Assumption

1. The sealant materials used for both treatment and control groups will be from the same batch, therefore the quality of the material would be consistent.

2. The operator and assistant who perform the sealant application in this study are highly trained and are the same persons throughout the study.

3. The examiner who evaluates the sealant retention is the same person throughout the study, hence the consistency of the intraexaminer reliability before and during the study.

Key words

Equivalence, pit and fissure sealant, retention, randomized, clinical trial

Conceptual Framework

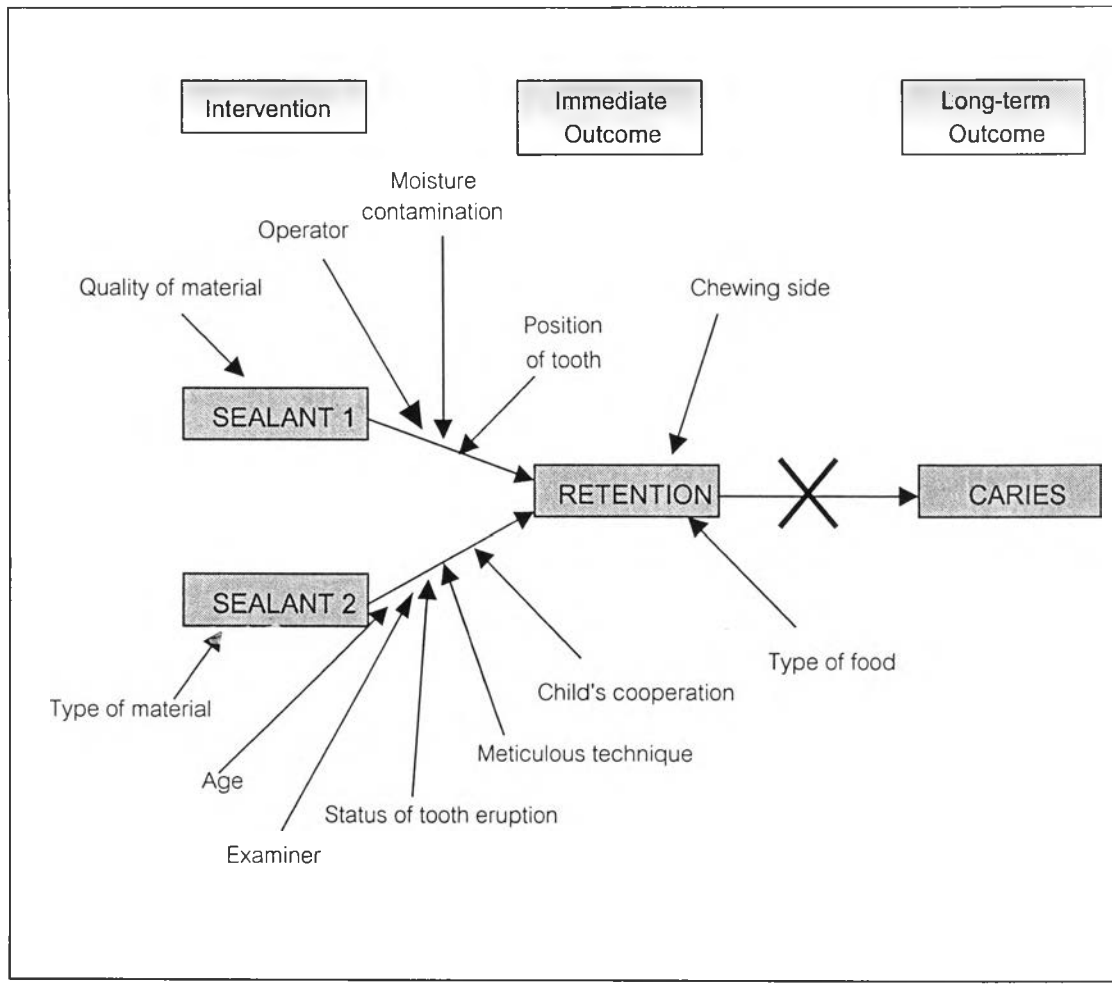

Figure 3.1 Conceptual framework

Operational Definition

1. Sealant retention

Sealant retention is the condition that the sealant material firmly adheres to the pit and fissure surfaces of the tooth. With visual examination, there should be sealant material present that covers the main pits and fissures at the perimeter of at least 1 mm from the pits or grooves on both occlusal and buccal surfaces. With tactile examination using an explorer, no dislogement of the material should be found.

2. Range of equivalence

The range of equivalence in this study is the sealant retention rates of 80-100% as determined by expert opinions from five pediatric dentists. Based on the acceptable sealant retention rate of 95% at 6 months interval (8), a relative difference of 10% in retention rates was a reasonable threshold to consider clinically important.

3. Dental caries

Dental caries is defined as any loss of tooth structure (enamel) detected by visual and tactile examinations. It is recorded according to the modified criteria of World Health Organization (65), by means of an explorer and a flat mirror.

Research Design

The study was carried out as a double blind randomized controlled trial. The aim of this study is to compare the clinical evaluation between the new local intervention (Chula Dent: Chulalongkorn University, Bangkok, Thailand) and the widely used, commercially available sealant (Concise™: 3M Dental Products, U.S.A.) in terms of retention on first permanent molars after 6 months. A matched pair experimental design was used, in which the test and the control sealants were randomly allocated to each side of the mouth. The blind process was applied to the intervention giver, patients, and outcome assessor.

Research Methodology

A. Population and Sample

1. Population

The population of this study is permanent molars of children that have never received previous sealant application.

2. Target Population

The target population is first permanent molars of children aged 6-9 years old eligible for sealing according to the indication for sealants (66).

3. Study Population

The study population comprises the first permanent molars of children aged 6-9 years old who attend Nongkae District schools, Saraburi province.

4. Sample Population

The sample population is the first permanent molars of children aged 6-9 years old who fulfill the eligible criteria.

B. Eligible Criteria

1. Inclusion Criteria

a) Children who had both lower first permanent molars with the following characteristics:
   (a) Both teeth should be caries free.
   (b) These teeth had not been filled or fissure sealed previously.
   (c) Deep pits and fissures.
   (d) Both teeth were sufficiently erupted to be free of the gingival tissue.

b) The two opposing upper first permanent molars were present and had normal occlusion.

c) Parents and their children gave informed consent and assent to participate in the study.

2. Exclusion Criteria

a) Children who were not cooperative.

b) Molars with enamel hypoplasia or dental fluorosis.

c) Dark stain on grooves or any evidence of decalcification (i.e. opacity, white spot) on the occlusal or buccal surfaces.

d) Molars with dissimilar anatomy on different side of the mouth.

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C. Sampling Method

Four schools in the district of Nongkae, Saraburi province participated in the study. The schools represented low to middle socio-economic backgrounds with high caries rate in the 5-6 year-old children. School selection was based upon accessibility, clinic availability and large school enrollment. Screening examination took place at three school sites by two dentists under well-lighted conditions using dental mirrors and explorers. Patient/ parental information sheet and informed consent were distributed to the parents or guardians. It was notified that eligible children would be invited to participate in a study at no charge.

D. Allocation

Children who fulfilled the eligible selection criteria and for whom the parents gave written informed consent allowing them to participate in the study were recruited into the study. Both lower first permanent molars of the same child were used.

Simple randomization using a computer-generated set of random numbers determined the assignment of each tooth to each sealant group. Therefore, each tooth had an equal chance of being assigned to either the intervention or the control group. The two sealants were randomly allocated to each side of the mouth, starting with the left mandibular first molar. The clinical procedures were performed during December, 2001 to February 2002 in the dental clinic of Nongkae District Hospital by a single operator who was a well-trained pediatric dentist and had high experiences in the use of fissure sealing with an aid of a chair-side assistant.

E. Intervention

The materials used in the sealant application process in this study were:

1. Treatment group:
    a. Chula Dent etching gel: (Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand, patented pending). Batch number 2000-8-10.
    b. Chula Dent sealant: opaque (Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand, patented pending). Batch number 2000-8-10.
2. Control group:
    a. Concise™ etching gel: (3M Dental Products, St. Paul, Minnesota, U.S.A.) Batch number 20010622.
    b. Concise™ Light Cured White Sealant: opaque (3M Dental Products, St. Paul, Minnesota, U.S.A.) Batch number 20010425.

F. Concealment

A research assistant produced the randomization scheme and wrote a random number for each sample in an envelope. At the time of sealant application, another research assistant opened the envelopes to get the assigned intervention and dispensed the materials for the operator. The experimental sealant was visually similar in color (opaque white) to the control sealant and the handling of both materials was also identical. Hence the operator and the evaluator did not know the materials being used. The patients were not informed as to which arm of randomized grouping they were in. All the codes were revealed at the time of analysis, i.e. after 6 months.

G. Exit from Protocol

The patients would exit the protocol in one of the following circumstances:

1. Upon completion of the protocol, i.e. the measurement of the primary outcome was complete.

2. The patient had severe adverse reaction before the completion of the protocol, e.g. allergy to the materials.

3. The patient/ parents decided to withdraw from the study.

H. Sample Size

This study compared two products that contained similar major components. The calculation for sample size was one suitable for equivalence trial. In the confidence interval approach, equivalence is concluded if the interval falls entirely within two prespecified tolerance limits (-10 to 10% difference or equals to retention rates of 80 to 105% in this study). According to Jones et al. (64), the formula used to estimate the sample size in a two-sided equivalence study with binary data is:

$$n \text{ (pairs)} = \frac{2p(100-p)}{\Delta^2}\left[Z_{(1-\alpha)} + Z_{(1-\beta/2)} \right]^2$$

p	= overall percentage of success	= 95 %
Δ	= difference in percentage success rates	= 10 %
α	= 0.025 (type 1 error)	
β	= 0.20 (type 2 error)	

P is the overall percentage of successes to be expected if the treatments were equivalent and delta (Δ) is the range of equivalence in percentage success rates, i.e. the minimum difference considered clinically important.

From previous studies, the retention rates of sealants at 6 months were 90-100% (8, 33). Hence the estimate of success rate (p) of 95%. The choice of delta requires extensive debate with knowledgeable clinical experts (64). Five pediatric dentists expert in the field were inquired to define this acceptable boundary. All of them unanimously agreed that a relative difference of 10% in retention rates was a reasonable threshold for replacing a standard sealant with an innovative one. Thus, the

predetermined level for the minimal difference considered clinically important for this study, i.e., $\triangle$ was 10%.

Two materials used as pit and fissure sealants were assessed for equivalence. A new sealant would be considered equivalent to the standard material if the 95% two-sided confidence interval for the treatment difference fell wholly within the interval +10%, that is, $\triangle$ =10 and α =(1-0.95)/2.

This required a sample size of approximately 100 subjects per treatment group. In order to allow for an expected 20% loss of participants, this study included 120 children per group.

I. Control of Co-interventions

The control for balance of the two groups was achieved by randomization and blinding process. If imbalance of the two groups was noted, all co-interventions were to be recorded for adjustment of the co-variates.

Instruments

Instruments during different visits were as follows:

A. During Sample Selection Visit

1. Oral examination set (a tray, a mouth mirror, an explorer no.5, a cotton plier)
2. Cotton rolls and gauze
3. Portable dental chair with light
4. Initial case record form
5. Parent information sheet and informed consent

B. During Sealant Application Visit

1. Oral examination set
2. Dental unit with high power suction, triple syringe and a prophylactic handpiece
3. Pumice powder
4. Rubber cup

5. Cotton rolls and gauze
6. Curing light unit (XL3000, 3M Dental Products, U.S.A.) that emits blue light at 470 nm wavelength and equipped with 10 mm diameter light tip
7. Concise™ White sealant set (3M Dental Products, U.S.A.) The set comprised etching gel and sealant material. The material is an unfilled visible light cured resin.
8. Chula Dent pit and fissure sealant set: etching gel and sealant material (unfilled visible light cured resin)
9. Paint brush (No.0, Saga Mayura, Bangkok, Thailand)
10. Steel finishing burs (round and flame shape)
11. Articulating paper
12. Randomization envelopes

C. During Evaluation Visit

1. Oral examination set
2. Portable dental chair with light
3. Cotton rolls and gauze
4. Case record forms

Method

A. Sealant Application

The standardization of procedures is as describe. All sealants were placed by one operator with the same assistant in order to keep the technique as uniform as possible. On seating the patient, the time was noted and the procedure timed to completion. For each molar, two discrete surfaces per tooth were eligible for sealant placement: the occlusal surface and the buccal surface. These surfaces were noted as different sites since they were not connected with each other. The procedure for the placement of both sealants was as follows.

1. Cleaning

The operator cleaned the teeth thoroughly with a fluoride-free mixture of pumice and water and a rubber cup in a slow-speed handpiece. With the aid

of an explorer, the operator carefully made sure that no plaque was present on the pits or grooves before rinsing and air-drying the tooth.

2. Tooth isolation

Moisture was controlled with cotton rolls, saliva ejector and a high vacuum suction. No rubber dam was used for isolation.

3. Surface preparation

Teeth were etched with an etching gel (37% phosphoric acid) for 15 seconds. A dental explorer was used to scrape the pits and fissures during etching in order to promote penetration of the acid into the fissures. At the end of the etching period, the tooth was rinsed thoroughly for 20 seconds with copious amount of water, and then dried with oil-free compressed air. The cotton rolls were changed after washing and drying with great care to prevent salivary contamination of the etched surfaces.

4. Sealant application

The sealant material was applied using a small paint brush (No.0, Saga Mayura, Bangkok, Thailand) to all parts of the pit and fissure.

5. Light curing

All sealants were cured with visible light (XL3000, 3M Dental Products, U.S.A.) for 20 seconds each on occlusal and buccal surface. The curing light was checked before each use for output using a curing radiometer (model 100, Demetron Research Corp.) The curing light intensity was found to be above 375 milliwatts/centimeter^2, which surpassed the acceptable output. After the placement was finished, the operator tested the primary retention of the sealant by applying dislodgement forces with an explorer.

6. Adjustment

On completion of each sealant application, the subject's occlusion was checked for any interference which might arise, and any adjustment necessary made with a flame shape finishing bur.

B. Follow-up exam

Six-month follow-up examinations were conducted to determine sealant retention rates and the existing of dental caries by one examiner who did not take part with sealant application. All follow-up examinations were performed at the participating schools, using a portable dental chair, head lamp, mirror, and explorer. The examination included the cleaning of debris and plaque from the teeth by using an explorer and gauze or cotton, and drying of the teeth using an air syringe attached to a portable air compressor.

In instances where partial or total loss was recorded, the tooth was excluded from further assessment. Sealants with a failure score were repaired by means of the same materials as on the original sealant application.

Measurement

A. Variables

1. Independent variable

The independent variable is the intervention given, i.e., the pit and fissure sealants.

2. Dependent variable

The dependent variable is the sealant retention at 6 months.

B. Primary Outcome Measurement

1. Location of sealant measurement

In all teeth, two fissure surfaces were defined (Figure 3.2):

a. The occlusal surface

The occlusal surface comprised three main pits, which were mesial (M), central (C) and distal (D) pits and the central groove that connected them. The accessory fissures were not included in the outcome assessment.

b. The buccal surface

The buccal surface comprised a buccal pit (B) and a groove that continued from the occlusal surface. The occluso-buccal line angle separated the occlusal surface from the buccal surface.

Figure 3.2 Location of measurement in a lower mandibular first molar

A. Occlusal surface B. Buccal surface

2. Sealant retention

Clinical evaluation was performed using the visual-tactile method (mirror and explorer). The sealants had to be present visually and an explorer was used to detect a marginal degradation of the occlusal sealant.

Sealant retention was classified according to modified established criteria (40).

a. Complete retention

The sealant covered the whole fissure system. No marginal disintegration could be seen or detected with a probe and no caries found. With visual examination, there should be sealant material present that covers the main pits and fissures at the perimeter of at least 1 mm from the pits or grooves on both occlusal and buccal surfaces. With tactile examination using an explorer, no dislodgement of the material should be found. If some periphery fissures were uncovered following sealant wear, but no ledges were present, the sealant was classified as completely retained.

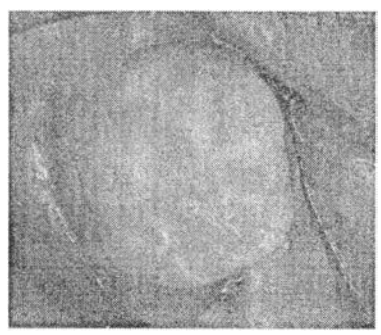
b. Partial loss

A sealant clinically absent from some of the pits or fissures. The sealant was present, but as a result or either wear or loss of the material. Part of a previously sealed pit or fissure, or both, was exposed. Loss of part of the sealant could be visualized or detected with an explorer.

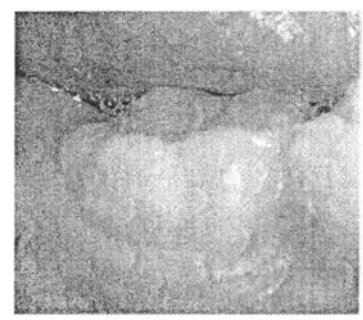
c. Complete loss

Sealant was undetectable visually or with an aid of an explorer in any pits and fissures.

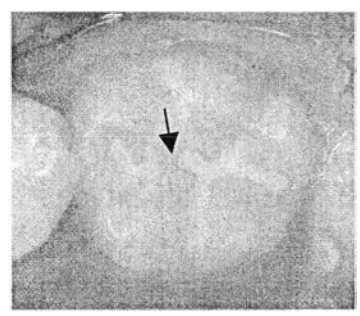
Figure 3.3 Criteria for evaluation of sealant retention



A. Complete retention on occlusal surface



B. Complete retention on buccal surface



C. Partial loss on occlusal surface

3. Scoring

a. Scoring of sealant retention according to each surface

a) 0 = complete loss

b) 1 = partial loss

c) 2 = complete retention

b. Scoring of sealant retention when the tooth was the unit.

a) 0 = failure

b) 1 = success

The clinical determination of sealant failure (score 0) was based on the sealant attaining a score of "complete loss" or " partial loss" as well as the presence of dental caries associated with pit and fissure system.

C. Control Measure for Reliability of the Data

The examiner was assessed for the intraexaminer reliability with respect to the recording of sealant retention. At the recall examination, the evaluator examined 25 children (only one molar from the same child) with respect to retention status of the sealant. Kappa of at least 0.8 was accepted for good agreement (67). The intraexaminer reliability was 100%, indicating excellent agreement.

D. Data Collection

Demographic data was completed by a dental assistant who filled up the data record form (Appendix 1). Outcomes for retention of the sealed teeth and the location of sealant loss were assessed and compared at six months after application by one evaluator other than the operator.

The data collection form comprised the following information:

1. Demographic data
   a. Name
   b. Date of birth
   c. Sex

d. School

2. Retention (primary outcome)

a. Success (score 1): defined as complete retention

b. Failure (score 0): defined as either complete loss or partial loss.

3. Location of sealant loss (secondary outcome)

a. Retention

The scoring for retention is as follows:

0 = Complete loss (CL)

1 = Partial loss (PL)

2 = Complete retention (CR)

The score 2 will be defined as "*success*" and scores 0 and 1 will be collapsed into the "*failure*" category.

b. Location of sealant loss

The scoring for location is as follows:

1 = mesial pit

2 = central pit

3 = distal pit

4 = buccal pit

Figure 3.4 Location of sealant loss by surface

A. Occlusal surface B. Buccal surface

4. Dental caries (secondary outcome)

Caries diagnosis was recorded according to the criteria of the World Health Organization (65), by means of an explorer and a flat mirror (Table 3.1).

Table 3.1 Criteria used for diagnosis of caries

Diagnosis	Code	Criteria
Sound	0	A crown is recorded as sound if it shows no evidence of treated or untreated clinical caries. No cavitation is detected.
Questionable	1	The stages of caries that precede cavitation, as well as other conditions similar to the early stages of caries, include white or chalky spots, discolored areas that are not soft to touch, stained pits and fissures that do no have visual signs of cavitation.
Caries	2	Caries is recorded as present when a lesion in a pit and fissure has an unmistakable cavity, undermined enamel, or a detectably softened floor or wall. A tooth with a temporary filling is included in this category.

Ethical Consideration

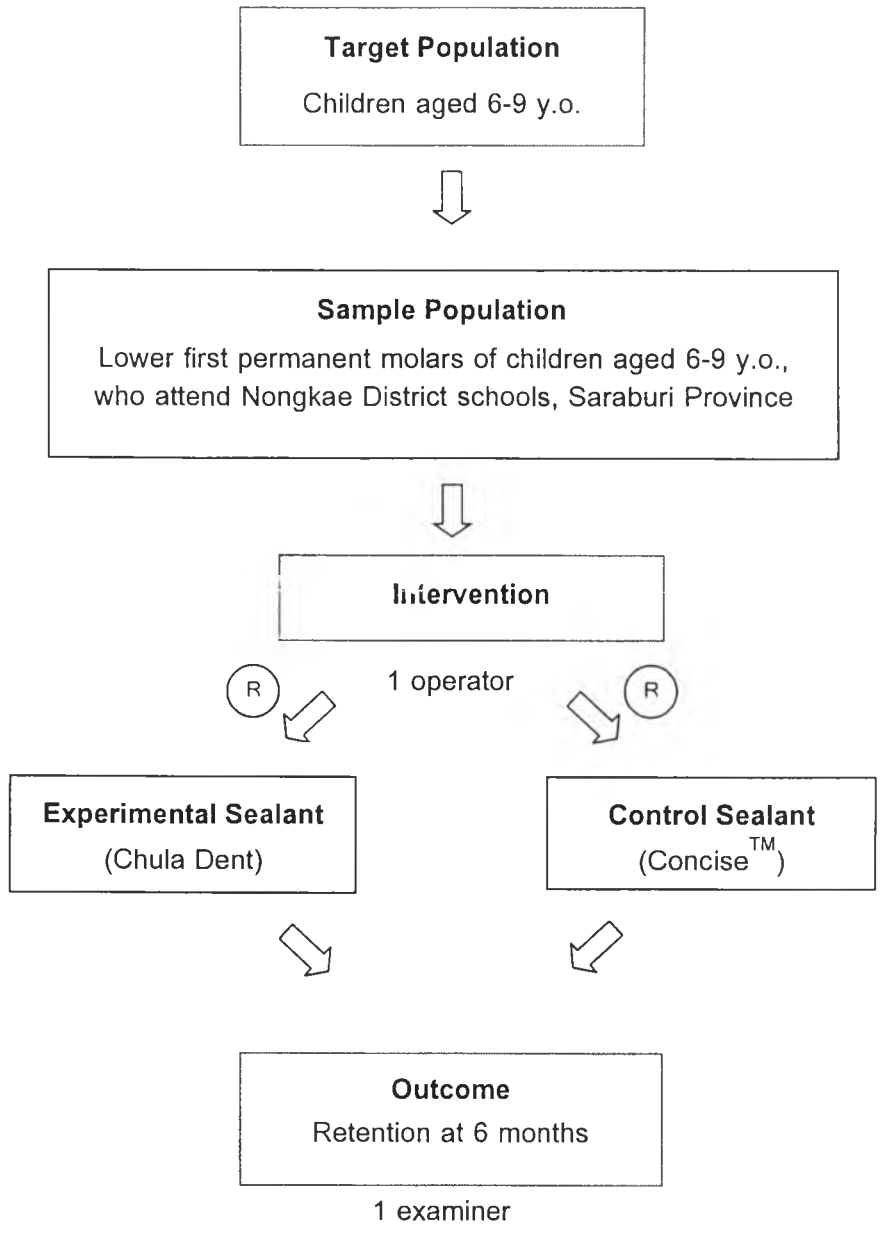
The study protocol was thoroughly explained to the parents and the patients. Permissions were obtained to participate in the study. The informed consent stated the risks and benefits of entering the study, and the parents were free to withdraw their child from the study at any time without affecting the quality of care.

The material of interest, Chula Dent, was similar in compositions to the standard sealant. In addition, physical, mechanical and biological tests according to the ISO standards showed that the material of interest was compatible to the conventional one.

In case of partial or total sealant loss, the same material would be reapplied to the tooth. If the tooth developed caries, the patient would be referred for appropriate care.

The protocol and details of the study were submitted to the Human Ethics Committee of the Faculty of Medicine, Chulalongkorn University for approval. It was approved on the 26th July, 2001. The number of study protocol approval form was 119/2001.

Figure 3.5 Diagram of study design



R = randomization

Statistical Analysis

Data were entered and verified (analyzed) using the computer statistical packages SPSS for windows (version 10.0) and STATA program (version 6.0).

A. Demographic variables

The baseline demographic data of the two groups (age, school and sex) was presented by descriptive statistics, using the mean and standard deviation for continuous data and percentage for categorical data as shown in Table 3.2.

Table 3.2 Baseline demographic variables and statistics used

Variables	Type of Variable	Statistics
Age	Continuous	Mean, S.D.
School	Categorical: nominal	Percentage
Sex	Categorical: dichotomous	Percentage, ratio

B. Outcome variables

The primary objective of this trial is to determine whether the effectiveness of a new material, Chula Dent, as measured by its retention onto a tooth, is at least equivalent to that of a standard material, Concise™ White Sealant.

The units of analysis in this study are the tooth and tooth surfaces of the lower first permanent molar (2 teeth and 4 tooth surfaces per child: mesial, central and distal pits of the occlusal surface and buccal pit of the buccal surface).

The sealant retention of both experiment and control sealants (number and percentage of teeth and sites of loss) were presented by descriptive statistics.

Difference in retention rates of both groups was compared by the calculation of 95% CI. The details of the statistical analysis are presented in Table 3.3.

Table 3.3 Outcome variables and statistics used

Variables	Type of Variable	Statistics
Sealant retention	Dichotomous (success, failure)	Descriptive Mc Nemar's Test 95% CI of difference
	Ranked order (complete retention, partial loss, complete loss)	Percentage
Location of sealant loss	Categorical: nominal (mesial, central, distal and buccal pits)	Percentage
Caries	Dichotomous (sound, questionable, caries)	Percentage