Regenerative Endodontic Therapy (RET)
using Antibiotic pastes or Calcium Hydroxide disinfection for the management of immature non-vital permanent teeth in children:
A Randomized Controlled Clinical Trial

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1.0 Background

The management of immature non-vital teeth following trauma or pulpal infection secondary to caries or dental anomalies e.g. fractured dens evaginatus tubercles is a challenge for dentists. Traditionally, the treatment prescribed for immature non-vital teeth is to thoroughly disinfect the root canal system and perform apexification procedures using materials such as $Ca(OH)_2$ or Mineral Trioxide Aggregate (MTA), followed by filling the root canal with gutta-percha. This technique however does not produce increased thickness of dentine or gain in root length to the immature tooth. Furthermore, in a retrospective study by Cvek, the risk of cervical root fractures has been found to be markedly higher in immature teeth treated with $Ca(OH)_2$ apexification and gutta percha, and its frequency was related to the stage of root development (1). This is possibly attributed to the fact that long term placement of calcium hydroxide as an intracanal medicament can induce brittleness of the tooth structure due to its hygroscopic & proteolytic properties, thus rendering them prone to root fracture (2). This emphasizes the importance of seeking other treatment approaches. More biologically compatible options, which offer regenerative possibilities and continued development of tooth structure have been explored, but are largely reported only in case reports and cases series.

Pulp tissue in immature teeth has the potential for regeneration following pulpal damage because they have wide open apices that are rich in blood supply and potentially viable apical tissues. Given non-infectious conditions, Regenerative Endodontic Therapy (RET) includes treatments such as revascularization via blood clotting technique, postnatal stem cell therapy, pulp and scaffold implantation, injectable scaffold delivery, three-dimensional cell printing, and gene delivery(3). All have the potential to provide revolution in endodontic therapy as it offers patients an alternative method to save teeth that have compromised structural integrity and poor long-term prognosis. Revascularization via blood clotting aside, the other RET techniques mentioned are designed on the accepted principles of tissue engineering, and aim to harness the potential of stem cells in the apical papilla through the provision of a sterile environment, signaling molecules and a scaffold within the root canal of the non-vital tooth (4). However, the use of advanced tissue engineering techniques for dental tissue regeneration, although promising, is still only at a laboratory and animal trial stage(4).

The advantages of RET in young patients include better long term prognosis of teeth due to continued root development and thickening of lateral dentinal walls; possible occupancy of root canal systems by vital tissues; and shorter treatment time with potential reduction in treatment fatigue. Case reports on revascularization via blood clotting technique (5-9) have shown that immature non-vital teeth can demonstrate continued root development under favourable conditions, which promote healing of apical

pulp tissue. Other case series (6, 8, 10) showed similar outcomes of continued maturation of root apices in teeth which had previously developed extensive periradicular lesions with sinus tract formation prior to treatment. It is essential to note that in some of these cases, mechanical instrumentation was not employed, and disinfection of the root canal was achieved purely through chemical means of disinfection using irrigation with sodium hypochlorite and application of antibiotic pastes.

These findings demonstrate that vital pulp rich in regenerative potential is present at the most apical portion of the root, and that immature non-vital teeth with periradicular pathology can still undergo apexogenesis in the presence of a generated sterile environment. Findings also indicate a paradigm shift of endodontic treatment away from predominantly restorative philosophy with filling of available canal spaces towards a biological and regenerative approach.

Status of scientific evidence on RET

As of current status, most of the evidence present on RET remain at case report and case series levels. While the multitude of publications to date appear to suggest that RET can lead to positive outcomes (e.g. healing of apical periodontitis, continued radiographic root development, and improved tooth survival), the success of this technique should still be taken with caution since case reports/series in nature are predisposed to publication bias with primarily positive outcomes reported. According to the American Association of Endodontists statement on regenerative procedures (11), there are currently no evidence based guidelines to support a standardized protocol, which provides the most favorable outcome in the treatment of infected immature permanent teeth. Various groups have in the course of their work made modifications to the procedures reported in the literature.

There are currently only two randomized controlled trials currently published in the literature. (12, 13) Nagy et al compared radiographic healing of apical periodontitis following MTA apexification versus RET procedures, in which the triple antibiotic paste was used as the intra-appointment medicament. They found that RET procedure allowed the continued root development in necrotic teeth with evidence of healing in some but not all cases, suggesting that healing outcomes using RET method has yet to be consistent or predictable.(13) Nagata et al found that the although the outcomes of patients with traumatized teeth treated with triple antibiotic pastes vs combination of calcium hydroxide and 2% chlorhexidine gel had similar clinical and radiographic outcomes, only 5 teeth (41.7%) and 3 teeth (27.3%) respectively demonstrated an increase in root length.(12) On the whole, the sample sizes in both these studies were very small at 12 patients per group, and the clinical outcomes and success rates shown were not convincing of the benefit of this treatment protocol. There is without doubt a paucity in well documented long term prospective clinical studies to show evidence on the success and survival rates of

teeth treated with RET, with many aspects surrounding the predictability of this procedure and true nature of treatment outcomes remaining unanswered.

Various intra-canal medicaments have been reported for use as root canal disinfectants with successful clinical outcomes. The feasibility is supported by controlled animal studies and case reports, in which the combination of ciprofloxacin-metronidazole-minocycline (14), or "3–Mix" paste as it is commonly known, at present is most well documented to be effective in disinfecting immature teeth with apical periodontitis and inducing apical closure in these infected teeth. However, a known drawback is discolouration of the tooth likely to be due to the minocycline component of the antibiotic mixture (15). To circumvent this problem, other combinations of intracanal medicaments for root canal disinfection have been proposed and reported in the literature. These include $Ca(OH)_2$ (16, 17) and different combinations of antibiotics preparations (18, 19), all which show comparable clinical success rates to the 3-Mix preparations. Having said that, the quest for an intracanal medicament with the most reliable clinical outcome is still underway.

This also begs the question if antibiotics are truly a necessary and beneficial component in the preparation for a sterile root canal. Prior laboratory in-vitro studies have determined that disinfection protocols have a profound effect on stem cells of the apical papilla (SCAP) as well as dentine microhardness. Yassen et al demonstrated that dentine microhardness was significantly reduced when triple or double antibiotic mixtures were used for treatment of dentine compared to $Ca(OH)_2$ (20). Antibiotic paste formulations used in RET procedures were found to be directly toxic to stem cells even at concentrations as low as 1% of doses currently used in treating patients, while conversely dentin conditioning with Ca(OH)2 was found to promote SCAP survival and proliferation (21, 22). This may well have clinical implications impacting fracture resistance and SCAP survival in immature permanent teeth with already compromised root thickness.

Given that this procedure (Regenerative Endodontic Therapy using revascularization via blood clotting method) has been carried out in children in Singapore since 2007, and a retrospective study is in progress, it is considered timely to conduct a prospective randomized controlled study to evaluate the need for antibiotic pastes and the outcome and success of RET.

2.0 Research questions/Aims and Objectives:

Research questions:

1. Are there differences between treatment outcomes of a 2-Mix antibiotic paste (Ciprofloxacin and Metronidazole) vs disinfection with the currently accepted multiple-visit protocol in non-surgical root canal treatment (i.e. disinfection with NaOCl solution followed by $Ca(OH)_2$ as inter-appointment dressing) with respect to:
    i. Resolution of apical periodontitis.
    ii. Thickening of dentinal walls and/or continued root development with or without apical closure.
2. Which are the baseline characteristics which could predispose a case to successful outcome?
3. Will discolouration of teeth occur and to what extent?

Primary aim

1. To assess frequency of periapical healing in the two sets of experimental protocols.
2. To study the differences in qualitative and quantitative increase in root dimensions of different protocols, by evaluating continued root development and thickening of dentinal walls.

Secondary aims

1. To evaluate if tooth discolouration occurs by evaluating the changes in tooth colour following treatment using the different protocols.
2. To evaluate patient reported outcomes, including oral health-related quality of life (OHRQL), inter-appointment symptoms, and satisfaction ratings.

Hypothesis:

1. There is no difference between treatment outcomes of RET using 2-Mix antibiotics (Ciprofloxacin and Metronidazole) vs disinfection with the currently accepted multiple-visit protocol in non-surgical root canal treatment for RET procedures in non-vital immature permanent teeth in children.
2. There is no difference between patient related outcomes of RET using 2-Mix antibiotics (Ciprofloxacin and Metronidazole) vs disinfection with the currently accepted multiple-visit protocol in non-surgical root canal treatment for RET procedures in non-vital immature permanent teeth in children.

3.0 Materials and Methods

3.1 Study Design

This will be a multi-center, parallel-group allocation (1:1), randomized controlled trial of treatment carried out on non-vital immature permanent teeth in children using revascularization via blood clotting technique. Both the patient and the assessors will be blinded to the intervention. The clinical study will be carried out at the School Dental Service, Health Promotion Board and NUH Dental Centre over a period of 3 years.

Regenerative Endodontic Therapy (using revascularization via blood clotting method) will be defined as any permanent tooth which has undergone at least a two-stage procedure involving:

a. Disinfection using sodium hypochlorite irrigation and placement of inter-appointment dressing (antibiotics or calcium hydroxide), followed by
b. Procedures which involve inducing bleeding and blood clot and hence intentional initiation of proliferation of stem cells of the apical papilla (SCAP) into the root canal.

3.2 Intervention: Study arms and Investigational Product (IP) used

Following canal debridement procedures, patients will be randomly assigned to receive different combinations of inter-appointment dressing, which will be one of the following:

- Experimental: Group A - Double Antibiotic Paste consisting of Ciprofloxacin and Metronidazole
  Intervention named: 2-Mix Antibiotic Paste
- Experimental: Group B - Non-setting calcium hydroxide paste inter-appointment dressing, aimed at prevention of re-colonization of canal space.
  Intervention named: $Ca(OH)_2$

3.2.1 Preparation of Antibiotics

Group 1 - 2-Mix Antibiotic Paste: This will be prepared at a 1:1 ratio of Ciprofloxacin 500mg (Tablet) and Metronidazole (Tablet) 400mg. To be crushed in a pill crusher immediately prior to administration. The antibiotic powder will be mixed with 3-4 drops of sterile water, to consistency of MTA/wet pumice.

Group 2 - $Ca(OH)_2$: This will be $Ca(OH)_2$ paste placed into the root canal according to currently employed procedures.

3.3 Outcome measures

The study will evaluate the following clinical outcomes following this procedure:

i. Resolution and healing of periapical radiolucency
ii. Measurements of thickening of dentinal walls and/or continued root development with or without apical closure
iii. Discolouration of tooth
iv. Patient reported outcomes, including oral health-related quality of life (OHRQL), symptoms, and satisfaction ratings.

3.4. Participants

3.4.1 Inclusion and Exclusion criteria

The inclusion criteria are as follows:

1. Patients aged 6-16 years of age
2. Patients who are fit and healthy or with ASA 1 or ASA 2 medical conditions
3. Patients with cooperation level that would allow treatment under local analgesia, application of rubber dam isolation and taking of intraoral radiographs.
4. Patients with permanent premolars that have incomplete root formation with an open apex of greater than 1mm width as observed radiographically and of single root canal morphology (23)
5. Patients with single rooted immature permanent teeth having one of the following pulpal and periapical diagnosis:
    a. Pulpal
        i. Necrotic pulp
        ii. Symptomatic and inflamed pulp not expected to heal. During attempted Cvek or cervical pulpotomy procedures, if the pulpal bleeding does not stop with direct pressure within 5 minutes, pulpectomy will be carried out and the patient will be recruited for RET procedures.
    b. Periapical
        i. Symptomatic apical periodontitis (with or without a radiographic apical lesion)
        ii. Asymptomatic apical periodontitis (with a radiographic apical lesion)
        iii. Acute apical abscess
        iv. Chronic apical abscess

Teeth will be deemed non-vital if either are non-responsive to sensibility tests (i.e. EPT and cold tests), and/or present with signs and symptoms of non-vitality (e.g. swelling and abscess).

The exclusion criteria are as follows:

1. Patients aged > 16 years of age.
2. Patient with known allergies to Ciprofloxacin (or any fluoroquinolones class of antibiotics) or Metronidazole antibiotics.
3. Patients with medical conditions and/or receiving medications that would affect:
    a. Their body's ability to heal e.g. Diabetes; or
    b. Their ability to clot efficiently, e.g. Haemophilia
4. Patients with risk of developing infective endocarditis or immune compromised patients.
5. Patients with non-vital permanent premolars where root development is already deemed to be completed (i.e. foramen size of 0-1.0mm diameter as determined radiographically).
6. Impacted or horizontally tilted teeth.
7. Concurrent signs of irreversible pathological root resorption determined radiographically, e.g replacement or internal root resorption, which could otherwise affect the prognosis of the tooth.
8. Uncooperative patients, or those unable to cope with treatment under local anaesthesia, rubber dam isolation and taking of intraoral radiographs.

3.4.2 Study settings

3.4.2.1 Recruitment of subjects and consent procedure

(A) Subject recruitment

About 3% of children in Singapore present with dens evaginations on their erupted premolars. The School Dental Service screens children aged 9-10 years (i.e Primary 3 and 4 pupils) for non-vital teeth as a result of fractured dens evaginatus tubercles as part of their annual screening protocols. The majority of potential subjects requiring treatment for non-vital immature permanent teeth will be identified through this screening.

Publicity will be carried out to heighten the awareness of the trial among dentists, and to increase the number of children recruited for the clinical trial. Advertisement emails through the Singapore Dental Association, dental specialist societies, and Oral and Maxillofacial staff at NUH will be distributed.

Treatment will be carried out at the following sites:

1. School Dental Service, Health Promotion board
2. National University Hospital – training and service sites

Clinicians rendering the revascularization procedures will be operators at NUH and School Dental Service, who are trained and already currently treating these cases under similar protocols. Prior to commencement of the study, all operators will be calibrated to the study protocol. All postgraduate student operators will receive standardized instructions and training for the treatment procedures (based on this protocol) at the beginning of their postgraduate course. Their training will be documented in the training and responsibility logs. Should postgraduates be rendering the revascularization procedures as part of their clinical training, they will be supervised at all times by experienced clinical instructors familiar with the treatment protocol.

Students will not be obtaining consent for treatment. Consent for treatment will be obtained by PI or co-investigators only.

(B) Consent procedure

If a patient is deemed suitable for inclusion in this study, parents and patients will be informed of the study by the primary attending dentist, and an Letter of Invitation (**Appendix A**) and information about the study (**Appendix B – Participant Information Sheet with Main ICF)** will be given to potential participants.

Potential subjects will be recruited by a face-to-face contact when they come for their next regular clinic visit. Only the PI and co-investigators will be involved in the consent taking. During the consent taking and consultation appointment, the investigator will counsel the parents/legal guardian and the child on the available treatment options for their condition. He/she will then explain the study to the parents and child.

The consultation session will be conducted in a private consultation room to ensure and protect the privacy of the subject from others' intrusion. The private consultation room will be a safe, closed environment in which the treatment options can be discussed with the parents/legal guardians and the child patient in confidence. The patient will not be recruited if the child declines to participate in the research despite parental consent.

When obtaining consent, the condition of the patient will already be stable and the patient should not be in pain. All emergency procedures would have been conducted in the previous visit, with pain addressed

in the previous session (prior to the recruitment session). Patients would have been given an information sheet about the study, and would hence have some time to read through and consider their options.

The attending dentist of the subject will not be involved in obtaining the consent of their own patient for research, so as to prevent the subject from feeling obliged to join the research, or have a heightened sense of faith and trust in their own physician, and may be more likely to participate. Another dentist who is part of the study team will be tasked to obtain informed consent instead. Possible languages that will be understood by prospective participants are English, Chinese, Malay and Tamil. In the situation that the investigator is unable to speak the language understood by the potential participant, a translator will be involved in helping to translate the information to the potential participant. The translator will be documented in the informed consent form. In this case, the participant will then be required to sign the DSRB short Consent Form (Translated).

Should the parent/legal guardian and child agree to participate in the study, they will be required to sign an Informed Consent form (parents/legal guardians) and assent form (child patient). Once informed consent is obtained, the operator will initiate the treatment.

Informed consent from the parents and child assent will be obtained. **Appendix B (Participant Information Sheet with Main ICF)** and **Appendix C1-4 (Chinese, Malay, and Tamil Short Consent Form and Child Assent form)**.

Participation in this study is purely voluntary. If either parents or patients are unwilling to participate in this study, they will not be recruited. Patients who wish to withdraw from the study may do so at any stage of the study. If the patient declines taking part in this study, the tooth will be accessed and dressed with $Ca(OH)_2$ according to currently accepted treatment protocol for management of patients with non-vital immature permanent teeth followed by application of MTA and obturation with gutta percha.

The data required will be collected prospectively. All data will be collected and analyzed in a de-identified format, with each individual assigned a unique identification number. IRB approval will be obtained prior to commencement of the study.

3.4.2.2 Intervention workflow (Appendix D)

Initial management of patients in pain:

Dental therapists and officers (who are the first line of clinicians managing emergency cases) will be instructed on the inclusion/exclusion criteria for the study. If a patient is deemed suitable for inclusion in

this study, they will be given an information sheet about the study and registered as a potential participant for the study. The treating clinician will seek the patients' consent to be referred to the study team and obtain their contact details which will be recorded and passed to the study team to schedule a consultation session. The consultation session and treatment will only be carried out by trained and calibrated operators. The estimated waiting period for consultation will be within 1 month of the initial diagnosis/emergency visit.

For patients who are in pain, dental officers will proceed to carry out emergency treatment for the patient, i.e. pulp extirpation/pulpectomy procedures, irrigate only with sodium hypochlorite solution, and seal temporarily with cotton pledget and IRM restorative materials. All procedures will be carried out under local anaesthesia and rubber dam isolation. This procedure is adequate to temporarily relief the patient from pain. Dental officers will also record the initial pulpal and periapical diagnosis of the tooth involved.

Subsequent visits: Patient contact and consent taking procedures

Potential subjects will be recruited by a face-to-face contact when they come for their next regular clinic visit. During the consent taking and consultation appointment, the operators (investigators/study team) will counsel the parents/legal guardian and the child on the available treatment options for their condition. He/she will then explain the study to the parents and child.

The consultation session will be conducted in a private consultation room to ensure and protect the privacy of the subject from others' intrusion. The patient will not be recruited if the child declines to participate in the research despite parental consent.

When obtaining consent, the condition of the patient will already be stable and the patient should not be in pain. All emergency procedures would have been conducted in the previous visit, with pain addressed in the previous session (prior to the recruitment session). Patients would have been given an information sheet about the study, and would hence have some time to read through and consider their options. The attending dentist of the subject will not be involved in obtaining the consent of their own patient for research, so as to prevent the subject from feeling obliged to join the research, or have a heightened sense of faith and trust in their own physician, and may be more likely to participate.

Should the parent/legal guardian and child agree to participate in the study, they will be required to sign an Informed Consent form (parents/legal guardians) and assent form (child patient). Once informed consent is obtained, the operator will initiate the treatment. The treatment will be carried out over 2 visits, each lasting about 1 hour per session.

For more information on treatment sequence, please see section on Randomization.

First Treatment Visit:

- The tooth will be examined and evaluated for clinical signs of an abscess, increased mobility and presence of the existing coronal seal restoration prior to commencement of local anaesthesia and rubber dam procedures.
- Pre-operative photographs will be taken by the operator. The photographic settings are as follows:
    - Camera: Canon EOS 600D
    - Shutter speed: 1/200
    - F stop: 32
    - ISO: Auto
    - White balance: Auto
- Treatment will be carried out under local anaesthesia and rubber dam isolation. Local anaesthesia with a vasoconstrictor may be used during this step.
- The tooth will be accessed and pulp necrosis will be confirmed. The root canal system will then be irrigated with 1.5% sodium hypochlorite.
- The canal will then be negotiated with minimal or no filing to prevent further weakening of the existing dentinal walls.
- 17% EDTA 10ml will be used to irrigate the canals.
- The canal will then be dried using paper points.
- Control or test procedure will be decided (**Appendix E - Patient ID, Intervention Allocation and Documentation Workflow**)
- The allocated antibiotics will be mixed with sterile water into a grainy mixture.
- The antibiotics will be placed into the root canal with a carrier and pluggers to completely fill the root canal system; Controls will be filled with $Ca(OH)_2$, placed in according to currently employed procedures.
- The root canal will then be hermetically sealed with a sterile cotton pledget, IRM and a glass ionomer cement to prevent any coronal leakage or contamination of the root canal with oral microorganisms.
- The patient will then be discharged, and given an appointment to return for the next step of treatment in 4 weeks ± 7 days.

Second Treatment Visit:

- The tooth will be examined and evaluated for clinical signs of an abscess, increased mobility and presence of the existing coronal seal restoration prior to commencement of local anaesthesia and rubber dam procedures.
    - If any of the above is present, a repeat session of disinfection is necessary and the first treatment visit procedures will be repeated until there is no clinical presence of an abscess or sinus tract. Information on number of additional disinfection visits will be recorded.
    - The operator/assistant will refer to patient ID recorded in treatment notes, and access the Intervention Log and double check the intervention group assigned to the patient prior to preparing the medicament to be used.
- Local anaesthesia without a vasoconstrictor will be administered and the tooth will be isolated and re-accessed as described above.
- The antibiotic mixture will be flushed out of the root canal by irrigation until solution runs clear with sodium hypochlorite. $Ca(OH)_2$ dressing will be removed by essentially the same procedure.
- The root canal will be irrigated slowly with 10ml 17% EDTA.
- Following this, the root canal will be thoroughly dried with paper points.
- A sterile 23-gauge needle or sterile file with a length of 25mm will be inserted beyond the working length and past the confines of the root canal into the periapical tissues to intentionally induce bleeding into the root canal. The bleeding is then allowed to fill the root canal.
- Once the root canal is filled with blood, a moist cotton pledget will be placed in the root canal below the cementoenamel junction (CEJ), and a clot will be allowed to form in the root canal.
- Once the clot is formed, the coronal portion of the root canal will be cleaned (with a new sterile cotton pledget moistened with saline) to remove any remnants of blood, which could cause discolouration in future
- Collagen will be placed directly onto the blood clot.
- The access cavity will be hermetically sealed with three layers of materials: Biodentine®, glass ionomer cement (Fuji IX) and composite resin. A post-op X-ray will be taken.
- Patients will then be reviewed at 3 months (± 2 weeks), 6months (± 2 weeks), 9months (±2 weeks), 12 (± 2 weeks) months, 15 (± 2 weeks) months and 18 (±2 weeks) months from treatment completion date. It would be acceptable for patient to miss one or more recall visits if patient comes for last recall visit at 18(± 2 weeks) months post treatment.

3.4.2.3 Review visits and parameters for evaluation of clinical outcomes

The primary purpose of review appointments is to assess healing and continued root development. Patients will be reviewed according to the set protocol stated above. Each tooth will be accounted for as an individual case, regardless of whether it originates from the same patient or not. Data will be recorded into a data collection sheet (**Appendix F**) and transferred into an Excel spreadsheet prior to analysis.

During the review visits, the following outcomes will be assessed:

Clinical-

- Presence/absence of tooth
- Mobility
- Periodontal probing (6 point evaluation for pocket depth)
- Tenderness to percussion and palpation
- Percussion note
- Free from pain or discomfort
- No evidence of labial swelling or abscess, signifying continued presence of infection
- Presence of discolouration

Radiographic

Standardised periapical radiographs will be taken before the start of treatment, at the end of the treatment and then again at each recall visit post treatment. Final outcome measures will be recorded at the 18th month review. This protocol is a standard clinical protocol and no additional radiographs will be taken for the purposes of this study.

A standardized beam aligning holder for both direct and indirect sensors will be utilized during taking of radiographs. All radiographs will be evaluated for periapical status and qualitative and quantitative status of root dimensions.

Standard Pulp Sensibility Tests

At each recall, pulp sensibility responses will be evaluated using thermal test (EndoIce) and Electric Pulp Test (EPT). Evaluation of tooth mobility, tenderness to percussion and palpation will also be carried out. This procedure will be done at the baseline, and during each subsequent review visit following completion of treatment.

Photography

Standardized intra-oral photographs will be taken before the start of the treatment and each recall visit. Post treatment crown colour in comparison to the pre-operative photographs and to that of the contralateral tooth will be assessed using the MathWorks software as described in the study by Day et al

(24). To facilitate comparison between pre-operative and post-treatment photographs and then quantify any color change, a dedicated software developed using MathWorks (Natick, MA) will be used. The software will be used to calculate the red-green-blue (RGB) and L*, a*, b* values for 2 areas of interest, after it had first corrected the color of the second photograph to that of the first. The program will evaluate the values for the reference (baseline) and final review photographs, together with the change in color, CIELAB ΔE.

Patient Reported Outcomes

For each session, the patients will be requested to evaluate their pain scores both pre-operatively (which will be related to the condition) and also post-operatively (related to treatment procedures). They will also be requested to fill in a patient satisfaction report which will evaluate the following outcomes: (1) Perceived improvement in oral health-related quality of life (OHRQL), (2) history of inter-appointment symptoms, and (3) overall patient satisfaction ratings. The questionnaires evaluating patient report outcomes will be piloted prior to use. (**Appendix G**)

3.4.2.4 Radiographic Analysis

Measurements of digital radiographs will be carried out using the measurement tool available in the VixWin digital radiography viewing software. Calibration of the measurements (based on the known width of the digital film) will be done prior to carrying out of quantitative measurements. Digital radiographs will be read in a darkened room, on a standardized monitor (DELL E2213H 1920 x 1080 Pixel Resolution 22" LCD Display) screen and computer with Windows Operating System (OS).

For length measurements, the point of reference will be the cement-enamel junction (CEJ) and Measurement ideals are referenced from the article by Bose *et al*(10), Chugal *et al* 2001(25) and Yu *et al* 2012(26). Below are the set definitions and proposed criteria which will be used for measuring the abovementioned parameters for radiographic analysis:

1. Size of periapical lesion,
   - Measured using the periapical index (PAI) scoring system (27)

2. Resolvement of periapical lesion;
   - Defined as the complete absence of existing periapical radiolucency as compared with pre-operative radiograph

3. Root length

- Quantitative outcome: Measured in millimeters (mm) as a straight line from the CEJ to the radiographic apex of the tooth;
- Binary outcomes: Comparison of increase in root length (Yes/No)
- Both pre and post-operative (measured during the recall visits) will be recorded.

4. Dentinal wall thickness measurements (at apical and mid root)
   - Quantitative outcome: Measured in millimeters (mm) at the apical one third of the tooth;
   - Binary outcomes: Comparison of increase in dentine wall thickness (Yes/No)
   - The dentinal wall thickness for both the preoperative and recall images will be measured at the level of the apical one third of the preoperative root canal length measured from the CEJ. The root canal width and the pulp space will be measured at this level, and the remaining dentin thickness will be calculated by subtracting the pulp space from the root canal width

5. Width of apex;
   - Measured in millimeters (mm) as the distance between the inner sides of the open apex
   - Binary outcomes: Comparison of decrease in width of apex (Yes/No)

6. Continued root development;
   - Measured as Postoperative root length minus Pre-operative root length (in millimeters) (mm); percentage change will also be calculated

7. Closure of apex;
   - Measured by means of visual identification; defined as approximation of the inner sides of the root tips.

8. Pulp canal obliteration
   - Defined as hard tissue deposited along the wall of the root canal which fills most of the pulp canal as detected through comparisons with pre-operative radiographs.

Since this study involves primarily young subjects (i.e. children), to account for possible clinical variations, both binary and quantitative outcomes will be collected. This is to account for clinical variations which may occur during the treatment process. The following parameters will be evaluated:

- Reduction in size or resolvement of periapical lesion
- Pulp canal obliteration
- Anomalies detected - any deviation from the norm, e.g. pulp canal obliteration, ingrowth of osteoid (bone)-like tissue etc.
- Continued root development and changes in dentinal root thickness and width of apex will be quantified using VixWin and Image J software.

Two independent assessors will be involved in the clinical and radiographic analysis. Prior to commencement of the analysis, the investigators will undergo training and calibration. All analysis will be carried out separately by the assessors, and results will be compared.

All radiographs will be randomly shown to the examiners who will be asked to score the test parameters. All available radiographs will be evaluated at the same time, and using the same computer under standardized monitor display configuration and lighting conditions. All measurements will be repeated after 1 week, and the mean of the 2 replicates will be considered as the final value. In cases where there is doubt or a large discrepancy between readings, both investigators will discuss and come to an agreement. All patient identifiers will be removed from the radiographs prior to radiographic analysis to prevent potential measurement bias.

3.5 Sample size calculation

This sample size is likely to be achievable given the expected number of cases that will be available in the clinics employed in this proposal. Retrospective data available from the School Dental Service, Health Promotion Board showed that RET treatment was provided to 78 patients (with 85 teeth) over a period of 3 years 6 months (Mar 2009 to Dec 2012). 77.2% of these teeth were mandibular premolars, 13.9% were maxillary incisors, and 8.9 % were maxillary premolars. Of these, 69.4% (n=59) had successful outcomes with closed apices following RET treatment, 7.1% (n=6) showed signs of increased root length and dentine wall thickening but were still undergoing review, 5.9% (n=5) did not show signs of root growth but demonstrated resolution of periapical pathology, 8.2% (n =7) had unfavourable outcomes with apices remaining open and signs of periapical pathology, and 9.4% (n=8) were loss to follow up.

The proposal will be for a sample size of 30 teeth per group, i.e. total estimated recruitment is 60 teeth. The sample size proposed is calculated based on data obtained from a published paper by Bose et al (2009). The sample size is estimated based on changes in root length as the primary outcome measure. Using a 2-sided sample test, assuming a mean difference between the groups of 1.5 and SD of 2, (at a significance level of 0.05 and 80% power), the sample size required is 30 subjects per group, i.e. 60 subjects in total. It is our hope that a multi-centre approach will help increase the caption group and thus increase patient numbers.

This is in agreement with sample size proposed in existing on-going randomized control trials which are registered on the Clinical Trial database website: www.clinicaltrials.gov. The clinical trials have similar age-range of potential patients as the proposed study.

4.0 Randomization

4.1. Sequence generation

It is proposed that the randomization process will be generated by a Biostatistician. A blocked randomized sequence will be used to generate a randomized sequence for each of the centres.

4.2. Allocation concealment and implementation

Randomization codes will be held by the delegated study team member, who will convey the randomization allocation to the operator. The delegated study team member (randomization code holder) will be responsible for maintaining the allocation log, and will inform the investigator of the assigned randomization ID as well as the intervention group assigned to the patient. **(Appendix E - Patient ID and Intervention Allocation and Log Documentation Workflow)**. The study team member who will perform the randomization will be trained and will be delegated in the responsibility log.

Materials to be used in both groups will be made available at each recruitment site. Potential participants must firstly be assessed for eligibility, be recruited and consented, after which only can the randomization codes be accessed.

4.3 Blinding and Unblinding protocol

The participants and the assessors (blinded team) for treatment outcomes will be blinded to the intervention used.

Unblinding will be done under the circumstances that any Serious Adverse Events (SAE) occurs during the course of the study, e.g. allergic reaction following administration of Investigational Product (i.e. antibiotics or $Ca(OH)_2$). All SAE or Unanticipated Problem Involving Risks to Subjects or Others (UPIRTSO) events will be logged and reported accordingly.

Unblinding Protocol: Unblinding envelopes containing the codes will be kept at the individual sites. In the event that unblinding will need to be carried out, the PI will access the codes to verify the investigational product used in the treatment.

4.4. Safety Reporting Guidelines

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or

Time frame and procedures for reporting of SAE to HSA will be in accordance to that specified by the Health Sciences Authority Health Products Regulation Group (Version June 2011).

For reporting of Unanticipated Problem Involving Risks to Subjects of Others (UPIRTSO), the following 2 criteria have to be met:

(A) Unexpected – Where in terms of nature, severity of frequency is not consistent with:

a. Research procedures described in the protocol related documents
b. Characteristic of subject population studied

(B) Related – where there is reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research

UPIRTSO reporting timeline for event:

1. Urgent Reporting: All problems involving local deaths, whether related or not, will be reported within 24 hours after first knowledge by the PI
2. Expedited Reporting: All other problems will be reported as soon as possible, but not later than 7 calendar days after first knowledge by the PI
3. HSA reporting requirements: The PI will inform HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. The PI will be responsible for providing HSA with follow-up information and to submit it as soon as it becomes available.

4.5. Statistical methods

All analysis will be carried at a tooth level, where each tooth will be accounted for as an individual case. Descriptive statistics (both discrete and non-discrete variables), which will include means, standard deviations, frequencies and percentages will be determined. Data will be checked for homogeneity of variance prior to application of statistical tests. Appropriate correlation and regression analysis will be

employed in consultation with a biostatistician. Inter and intra-operator variability scores will be evaluated using Kappa statistics. The significance level will be set at $p<0.05$. All data will be analyzed using SPSS Statistical Package (IBM SPSS Statistics).

5.0 Data management

5.1 Data storage

Any data collected on paper will be stored in a designated locked cabinet at the research site. Data will be entered into an OMR sheet for accuracy. All electronic data collected will be kept in a password secured portable computer and backed up to dedicated password protected local back-up drive every week.

Some measures may include password protection, security under lock and key, access controlled office, etc. To protect patient confidentiality, records and logs will be stored in locked file cabinets, in locked offices, and on computers protected by a password. A patient identification log will also be put in place - each patient's data will be coded with an identifier, and the key to the code will located in another physical location or on a separate computer. All patient identification logs will be kept for 6 years, after which it will be securely disposed of. The data will only be accessible to the study team members involved in and approved for the study. Identifiers of participants (i.e. birth certificate numbers, date of birth, email addresses, mailing addresses) will be extracted and each patient will be allocated an individual number to secure patient's identity. All data will be kept for 6 years upon completion of study.

5.2 Data Safety Monitoring

Data safety monitoring will be performed by the investigator and a team of co-investigators. Data at each site will be reviewed on a weekly basis by the PI. Data on adverse events/ serious adverse events will be monitored for safety of participants and reported accordingly.

Any data and safety information will be communicated to research members by the PI both verbally and by email circulation, and will be documented accordingly. The PIs will be responsible for doing Monthly or Adhoc meeting to discuss and update data and safety information with study team.

The PI or Co-Is will be responsible for verifying that the information entered into the data collection sheet is in accordance to what is in the clinical notes. Periodic monitoring of the study data collected will be monitored by a designated personnel who will be assigned to audit the study. This person will be trained by NUHS Research Office to conduct monitoring of clinical trial data recorded, and also compliance to SGGCP, SOPs and trial protocols and other regulations.

6.0 Potential difficulties and limitations

6.1. Issues with Recruitment

Recruitment of sufficient participants to reach adequate power for the study may be a challenge. We hope that a multi-centre approach will help increase the caption group and thus increase patient numbers. Furthermore, publicity will be carried out to heighten the awareness of the trial among dentists, and to increase the number of children recruited for the clinical trial. Advertisement emails through the Singapore Dental Association, dental specialist societies, and Oral and Maxillofacial staff at NUH will be distributed. A poster for recruitment of participants will be put up in designated rooms and waiting areas for each of the research sites. A copy of the Poster and Letter to the Singapore Dental Association for recruitment of participants are as in **Appendix H1 & H2**.

6.2. Ensuring patient return for reviews

As this is a randomized controlled clinical study, it is possible that patients may not return for recalls following completion of procedures, resulting in drop outs. As such, a 10% margin for drop outs has been assumed in the power calculation. Intention to treat analysis will also be carried out.

Patients enrolled in the study will be followed up closely, and appointments at appropriate intervals as specified in the protocol will be made. Patients will be reminded to attend the review visits 2 days in advance via telephone or SMS. Treatment rendered and review visits will not be charged.

6.3. Technical difficulties with radiographic analysis

With respect to radiographic analysis, there is a possibility of radiographic distortion and differences in angulations between pre and post-operative radiographs. We sought to minimize these errors by using a cone aligning holder. All available radiographs will be evaluated at the same time, and using the same computer under standardized lighting conditions. Calibration of radiographs using the computerized program VixWin and Image J (used to evaluate radiographic measurements) will be done prior to data analysis. The scale will be set for the digital films on the basis of the pixel size used in the VixWin digital system.

7.0 Timeline

- Start date: When DSRB and HSA CTC approval is obtained
- End date: (Proposed) Data collection to end by two years after last subject enrolled; closure by two and half years after last subject enrolled.

The study will be conducted according to all the principles of International Conference of Harmonisation-Good Clinical Practice (ICH-GCP) as well as the Singapore Guideline for Good Clinical Practice (SGGCP). The trial will be subjected to the regulations as stated in the Medicines Act and Medicine (Clinical Trials) Regulations. The trial will be registered with the appropriate institution review boards, as well as clinical trial databases both internationally (e.g. www.ClinicTrials.gov) and locally.

8.0 Impact of the study and Future directions

Traditional endodontic treatment in immature teeth do not have a good outcome as it has been shown that the failure rate of teeth treated using such approaches is very high (1). The use of RET has the potential to revolutionize the management of non-vital teeth with incomplete root development. Continued root development and thickening of the dentine walls of the root canals through regeneration method would mean that such teeth may survive for the lifetime of the patients.

An important aspect is that we will test the significance of the use of antibiotics. So far no study of this design has been conceived. Should the results of this study demonstrate that antibiotics are not required for achieving success in RET procedures, the need for antibiotics and hence risk of adverse drug reactions and emergence of resistance strains will be reduced. The streamlining of disinfection protocols for RET procedures will also make it easier for dental practitioners to perform this treatment for their patients.

Alongside the study on long-term outcomes and success rates of the two different protocols, this project will also study the patient reported outcomes (PRO), which is necessary to evaluate the young patient's perspective on treatment rendered, and has yet to be studied and reported in current literature. Results of the study will be presented at international conferences and published in relevant scientific journals. Given that only a few groups worldwide are working on this subject, such data generated from our study will contribute to knowledge on this subject globally and secure NUS/HPB an international impact in this field of clinical research.

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