Evaluation of Demineralized Dentin Matrix as a scaffold for Revitalization in immature permanent incisors: A Randomized Controlled Trial and In Vitro Study

A Project Submitted to the Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry Ain Shams University

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By

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I. Introduction

Necrosis of the immature pulp not only is one of the multiple factors affecting long-term tooth endurance and preservation, but also can act as a source of bacterial infection in periapical area and even the maxillofacial spaces [1]. The use of regenerative endodontic techniques (RET) have gained popularity in the past decade with several endodontic and pediatric dentistry organization recognizing this technique as a viable technique for immature permanent teeth with necrotic pulps [2,3]

Regenerative endodontics are defined as biologically based techniques planned to physiologically replace impaired tooth structure, including dentin and root constructions, as well as cells of the pulp–dentin complex [4]. They offer the benefit of root lengthening, thickening, and subsequent apical closure which is not achievable with conventional nonsurgical endodontic treatment (NSET). The success rate of the blood clot (RET) in immature teeth has been reported to be 97.8% [5-7].

The American Association of Endodontists, and European Society of Endodontology developed evidence based protocols. To help reducing some of the differences in the RET steps such as EDTA and sodium hypochlorite use, however, variations in terms of intracanal medicament, scaffold and assessment tools still exist due to the lack of high-quality evidence [8,9].
A fundamental component of the regenerative endodontic process is the presence of a scaffold for stem cells from the apical papilla to adhere, multiply and differentiate. Based on the results of many studies, the use of blood clot Regenerative procedure is not considered a true scaffold system. But it is an essential part of any appropriately designed tissue engineering regenerative endodontic strategy [10-12].

Different treatment options and scaffolds have been tested in regenerative endodontics. Only some of which exhibited clinical relevance such as blood clot, platelet rich plasma and platelet rich fibrin as RET scaffolds. Therefore, the use of suitable scaffold is an essential part of tissue engineering regenerative endodontic strategy [13-16].

Hence, developing a new dental material that retains the proteins or factors that can stimulate regenerative processes close to the natural process seems promising [17]. Tissue-derived extracellular matrices have recently been known as naturally-derived scaffolds for tissue regeneration in various applications and have been revealed to serve as a cultural substrate on which cells tend to adhere to and proliferate well and can induce regeneration specific to tissues and sites [18,19]

A new extracellular matrix material, demineralized dentin matrix (DDM) derived from extracted teeth has been found to act as a biocompatible scaffold for the attachment, differentiation, and proliferation of human DPSCs (dental pulp stem cell) into odontoblast-like cells [(20)]. Dentine matrix has proposed roles for directing mineralised tissue repair in dentine and bone; however, the range of bioactive components in dentine and specific biological effects on bone-derived mesenchymal stem cells (MSCs) in humans are less well understood [(21)].

DDM is autogenous tooth dentin that has osteoconductive and osteoinductive potential since it contains extracellular collagen-1 and various growth factors. DDM as a solid blocky material has less physical stability and sealing ability as a material for regenerative endodontics. Therefore it has been further fabricated into their forms of DDM powder, gel, and as sheets in order to maintain bioactivity [(22)].

DDM can be prepared with low risks of infection and rejection with noninvasive attainability; thus, it should be considered as a natural resource to be used to full advantage for other applications [(23)].

In 2022 a meta-analysis performed by **(Grawish et al., 2022)**[(23)] indicated that DDM as a regenerative biomaterial has potential roles in tissue regeneration. However, in order to confirm these trials, further well-designed analyses are needed, and evaluation of adverse events in observational studies is also needed.

To our knowledge, DDM has not been investigated as scaffold in regenerative endodontics for permanent teeth.

Several studies were evaluate the regenerative effect of DDM at dentin-pulp complex through direct pulp capping technique, and it was found to possess the ability to activate the odontogenic differentiation of stem cells resident in the pulp tissues and induce reparative dentin formation. DDM also considered for alveolar ridge, maxillary sinus floor augmentations, socket preservation, furcation perforation repair, guided bone, and bioroot regenerations as well as bone and cartilage healing [(23-27)].

Hence, this study will be designed to evaluate regenerative effect of Demineralized Dentin Matrix as a scaffold in comparison to conventional blood clot in immature permanent teeth.

II. The null hypothesis

The null hypothesis is that there are no significance differences in the clinical and radiographic outcomes of regenerative endodontic of non-vital immature permanent teeth following the use of DDM in comparison to a blood clot scaffold.

III. Aim of the study

This study aims to:
Compare the clinical and radiographic outcomes of demineralized dentin matrix scaffold to blood clot scaffold in immature permanent incisors with non-vital pulps for one year.

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IV. PICOTS

P: Patients ranging age 7–13years

I: Demineralized Dentin Matrix as a scaffold in Regenerative endodontic procedure

C: Blood clot scaffold Regenerative endodontic procedure

O: Clinical and radiographic success.

T: One year

S: outpatient's clinic of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University.

V. Methodology:

i. Study design

A Randomized, Controlled trial with 1:1 allocation ratio

ii. Sample size estimation

A power analysis was designed to have adequate power to apply a two-sided statistical test of the null hypothesis that there is no difference would be found between different groups. By adopting an alpha level of (0.05) a beta of (0.2) i.e. power=80% and an effect size (d) of

(1.51) calculated based on the results of a previous study [25]. the predicted sample size (n) was a total of (24) cases (i.e. 12 cases per group). Sample size was increased by 20% to compensate for possible dropouts during follow-up intervals to be (30) cases (i.e. 10 cases per group). Sample size calculation was performed using G*Power version 3.1.9.7[28]

iii. Assignment of intervention:

a. Randomization and Allocation concealment.

1-Type: block Randomization, with blocks numbers of 10 and1:1:1allocation ratio in to 3 groupsGroup I: DDM (study)
Group II: (control) blood clot
Group III: (+ control) blood clot with collagen

2- Sequence generation: the most senior supervisor will generate the sequence of random numbers using online randomization (www.random.org).

Allocation concealment and implementation:
Allocation concealment will be ensured by preparing opaque envelopes containing one number from the generated random sequence for assigned group. The most senior supervisor will select an envelope and reveal the treatment group at the final regeneration visit.

b.Blinding:

double blind study, participants, outcome assessors, statistician will be blinded.

iv. Study setting

This study will be conducted at the Outpatients Clinic at the Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Ain Shams University.

ii. Study methodology

ethical regulation:

The study protocol will be submitted to the Research ethics committee, Faculty of Dentistry, Ain Shams University for approval.

v. Population

All parent or legal guardian fulfill the eligible criteria will be signed an informed consent before beginning any procedure, explaining detailed treatment procedures, the possible outcomes, comprehensive discussions of the risks, complications, alternative treatment options, follow-up period needed, and sequel of no treatment [8-14].

Inclusion criteria:

(1) Age 7–13 years at the time of enrolment.
(2) Provision of informed consent by one parent or legal guardian.
(3) At least one immature permanent anterior tooth diagnosed with irreversible pulpitis or pulp necrosis with or without periapical lesions.
(4) Restorable teeth.
(5) Compliant patient/parent.
(6) Patients not allergic to medicaments and antibiotics necessary to complete procedure (ASA 1 or 2).
(7) Radiologic evidence of open apices (Teeth are considered immature when a minimum of 1 mm apical foramen width is evident)

Exclusion criteria

(1) Teeth with root fractures or split roots.

(2) Presence of root resorptions.

(3) Presence of periodontal pockets.

(4) Developmental anomalies

(5) Presence of periapical radiolucency more than 10 mm

(6) Tooth with class III mobility.

vi. Clinical Procedures

a. Screening Recruitment

Patients attending the outpatient's clinic of the Pediatric Dentistry and Dental Public Health Department, Ain Shams University will be screened for eligibility. Only patients who met the inclusion criteria and sign the informed consent will be conveniently enrolled in the study until

the predetermined sample size is reached. All examination procedures will be done by the same operator to ensure standardization.

b. Informed Consent

A signed informed consent from parent or legal guardian, and an oral assent to the child, as outlined by the Research Ethics Committee, Faculty of Dentistry, Ain Shams University will be obtained before the conduction of the study.

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Grouping:

Eligible immature anterior teeth will be randomly allocated in two groups:

Group I: 10 teeth will be treated with demineralized dentin matrix as scaffold in regenerative endodontic procedure.

Group II: 10 teeth will be treated with conventional regenerative endodontic procedure (blood clot scaffold).

Group III: 10 teeth will be treated with regenerative endodontic procedure (bloodclot and collagen scaffold).

Clinical procedures:

Pre-operative periapical radiograph and con beam computed tomography CBCT will be taken on the selected non-vital tooth in each child before the beginning of the regenerative endodontic treatment procedure [29, 30].

RET will be carried out according to ASE[8]

First Appointment

- Local analgesia will be delivered (2% lidocaine with 1:100,000 adrenaline).
- Following rubber dam isolation and access cavity preparation, pulp tissue extirpation/debridement
- The root canal system will be minimally instrumented.
- Copious irrigation will be performed, gently with 20ml NaOCl (1.5% NaOCl (20mL/canal, 5 min) using 0.3mm gauge single side vented needles, adjusted 3 mm short of the apex.
- then canal will be irrigated with EDTA (20 mL/canal, 5 min), with irrigating needle that will be positioned about 1 mm shorter than the root end, to minimize cytotoxicity to stem cells in the apical tissues, followed by a final rinse 20 mL sterile saline .

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- Canals will be dried with paper points.

double antibiotic paste, ciprofloxacin (Ciprocin 250mg tablets) and Metronidazole (Flagyl 500mg tablets) in a 1:1 ratio will be mixed with sterile distilled water in a 3:1 ratio, then will be introduced into the root canal systems using 18 G hypodermic needle and will be packed gently down the root canal using a hand plugger, ensuring that there will be no remains coronal to CEJ.

- A sterile dry cotton pellet will be placed over the paste after which the tooth will be sealed with 3-4mm of a temporary restorative material.
- Patient will be dismissed for 2week

Second Appointment (two week after 1st visit)

• Response to initial treatment will be assessed. If there are signs/symptoms of persistent infection, additional treatment time with antimicrobial.

• Local Anesthesia without vasoconstrictor will be administered; dental dam isolation will be achieved.

• Copious gentle irrigation will be performed with 20ml of 17% EDTA, followed by a final rinse 20 mL sterile saline.

• Canals will be dried with paper points.

In group I: teeth will be treated with demineralized dentin matrix as scaffold in regenerative endodontic procedure

Teeth, in this group, will be anaesthetised using a local anaesthetic without vasoconstrictor. Demineralized dented matrix will be introduced inside the root canal and bleeding will be created into root canal by over-instrumentation by rotating K-file at 2 mm past the apical foramen with the goal of having the entire canal filled with blood to the level of the cemento–enamel junction.

A lightly moistened sterile cotton pellet will be placed into the canal, 3-4 mm apical to the CEJ, for 7-10 minutes to allow blood clot formation.

Placement of the bioactive and restorative materials: A bioactive material such as tricalcium silicate will be placed over the blood clot as a capping material. A 3–4 mm layer of glass ionomer will be gently placed over the capping material.

Followed by resin composite restoration [16].

In group II: teeth will be treated with conventional regenerative endodontic procedure (blood clot scaffold).

Teeth, in this group, will be anaesthetised using a local anaesthetic without vasoconstrictor. Bleeding will be created into root canal by over-instrumentation

A lightly moistened sterile cotton pellet will be placed into the canal, 3-4 mm apical to the CEJ, for 7-10 minutes to allow blood clot formation.

Placement of the bioactive and restorative materials as mentioned above.

In group III: teeth will be treated with conventional regenerative endodontic procedure (blood clot and collagen scaffold).

Teeth, in this group, will be anaesthetised using a local anaesthetic without vasoconstrictor. Bleeding will be created into root canal by over-instrumentation

A collagen will be placed into the canal, apical to the CEJ.

Placement of the bioactive and restorative materials as mentioned above

e. Follow up

Clinical and Radiographic examination will be carried out during follow up period [15, 16].

1- **Clinical evaluation:** Treatment success will be assessed using following criteria of success.

Criteria of success: Absence of

- Pain
- Soft tissue swelling
- Soft tissue inflammation
- Sinus tract
- tenderness to percussion
- tooth mobility

2- Radiographic evaluation:

A-Conventional radiographic evaluation:

Criteria of success:

- Apical foramen width reduction
- Increased dentinal wall thickness
- Increased root length
- Conventional radiograph showing progress bone healing and presence of a normal periodontal ligament space.
- Reduction in size of the lesion.

B- CBCT evaluation:

- CBC with limited field of view will be carried out at 12 month to asses dentin width and length[23, 28]

f. Outcome measurement

Outcome	assessment	Assessment method
Primary outcome	1- pain 2-pain percussion 3-pathological mobility 4-gingival becket	1-visual assessment 2-percussion test 3-blunt ends of two examination mirrors 4-graduated metal periodontal probe
Secondary out com	1-Root length 2-Root dentinal thickness 3- Apical foramen width 4- Total radiographic root area (absence of internal and external root resorption, widening of periodontal ligament space, new preiabical radiolucencies or enlargement of pre-existing lesion, pulp canal calcification)	Digital periapical radiograph examination with using paralleling device
Secondary outcome	Increased root wall thickness and increased root length.	CBCT with limited field of view

VII. Statistical analysis

Categorical data will be represented as frequency (n) and percentage (%) and will be analyzed using chi square test. Numerical data will be explored for normality by checking the data distribution, calculating the mean and median values and using Kolmogorov-Smirnov and Shapiro-Wilk tests. If the data was found to be normally distributed, it will be presented as mean and standard deviation values and paired t-test will be used for the analysis. If the assumption of normality was found to be violated; the data will be presented as median and range values and will be analyzed using Signed rank test. The significance level will be set at $p \leq 0.05$ for all tests. Statistical analysis will be performed with IBM SPSS Statistics Version 26 for Windows.

VIII. Study timeline

This study will be conducted from October 2023 to October 2024.

X. Patient withdrawal

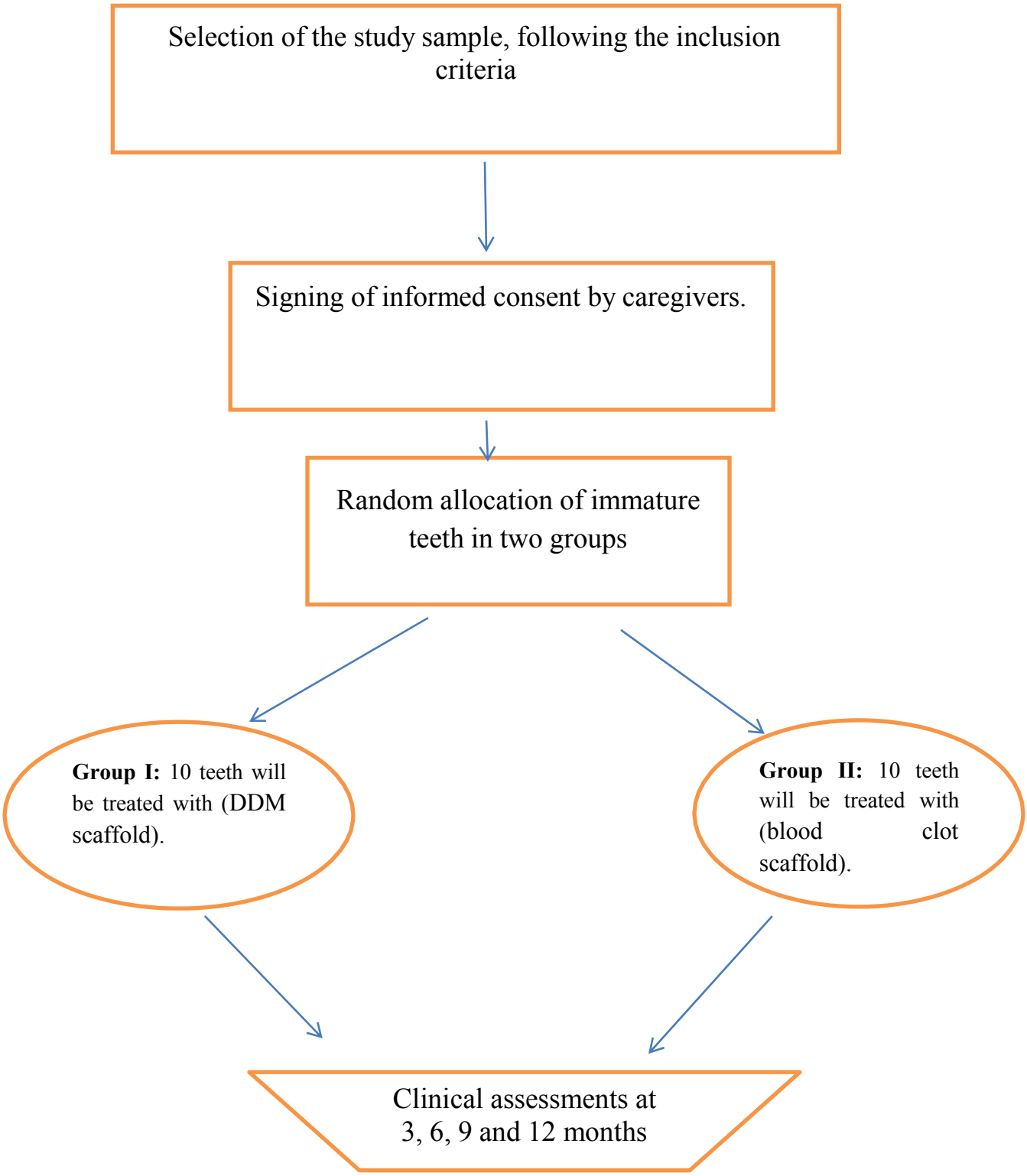
- Patients who aren't willing to continue in the study will have the right to quit at any time without loss of any benefits.
- Patients who don't comply with the oral hygiene measures given will be excluded from the study and will be offered to continue dental treatment at the departments' outpatient clinic.
- Any adverse event will be recorded and managed promptly. In addition, in failure occurs, the use of the experimental material will be stopped and patients will be treated with another modality.

xi. Data management

Patient information will be guarded as confidential information that should never be revealed at all times.
All information will be kept as a hard and soft copy.

xii. Adverse event reporting

Regenerative treatment is not expected to have any adverse side effects on health but if failure occurs in the regenerative treatment, conventional regenerative endodontic treatment will be fabricated.



Schematic presentation for the study design

xiii. Ethics and dissemination

a. Research ethics approval:

This protocol and the template informed consent form contained in Appendix are written following the guidelines outlined by the Research Ethics Committee and Institutional Review Board, Faculty of Dentistry, Ain Shams University.

b. Protocol amendments:

In case of protocol modifications, the Research Ethics Committee and Institutional Review Board, Faculty of Dentistry, Ain Shams University will review and approve the changes of protocol before proceeding and implementation of changes.

c. Informed consent:

A verbal as well as a written consent will be obtained from the caregivers of the participants after explaining the objectives of the study and assuring complete confidentiality of data. All caregivers and participants will be informed that they have the right to withdraw from the study at any time. Moreover, participants 6 years and older will be requested to sign an assent form after an age appropriate explanation of the trial procedures and their objectives. All consent and assent forms are translated into Arabic (APPENDIX I).

d. Confidentiality

All study-related information will be stored securely at the study site. Personal information, consent forms of enrolled participants will be collected by the primary investigator (candidate) and stored in hard copy examination sheets in locked file cabinets with limited access. All local databases will be secured on password-protected computers. Patients' identity will not be shared at any stage of the study. Participants receiving treatment will be given codes (identification numbers) for statistical analysis.

e. Access to data:

Primary investigator, supervisors and biostatistician will have access to the final trial dataset.

f. Post-trial care

All patients with premature loss of teeth in the patients' mouth will be treated with the proper treatment protocol. According to our knowledge, no side effects have been reported in literature from using both materials under study in patients with non-vital immature permanent teeth.

g. Dissemination policy

After completion of the study, trial results will be communicated to participants – if interested- via phone calls. Results, as well as some radiographs and clinical pictures of treated teeth, will be available for healthcare professionals and the public via publications.
Guidelines for authorship will follow the four criteria stated by the International Committee of Medical Journal Editors –ICMJE‖. Contributors meeting less than the four criteria will be acknowledged.

h. Declaration of interest

The investigators declare no conflict of interest.

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