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ORIGINAL ARTICLE

Efficacy of mixture of injectable-platelet-rich fibrin and type-1 collagen particles on the closure of through-and-through periapical bone defects: A randomized controlled trial

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Abstract

Aim: To determine the efficacy of a combination of injectable-platelet-rich fibrin and type-1 collagen particles on the healing of through-and-through periapical bone defect and subsequent closure of bony window.

Methodology: The clinical trial was registered in ClinicalTrials.gov (NCT04391725). Thirty-eight individuals with radiographic evidence of periapical radiolucency in maxillary anterior teeth and confirmed loss of palatal cortical plates in cone beam computed tomographic imaging were randomly assigned to either the experimental group ($n = 19$) or the control group ($n = 19$). A mixture of i-PRF and collagen as a graft was applied to the defect in adjunct to periapical surgery in the experimental group. No guided bone regeneration procedures were used in the control group. The healing was evaluated using Molven's (2D) and modified PENN 3D (3D) criteria. Percentage reduction of the buccal and palatal bony window area, and complete closure of through-and-through periapical bony window (tunnel defect) were assessed using Radiant Diacom viewer software (Version 4.0.2). The reduction in the periapical lesion area and volume was measured using Corel DRAW and ITK Snap software.

Results: Thirty-four participants (18 and 16 in the experimental and control groups respectively) reported for follow-up at 12 months. There was 96.9% and 97.96% reduction of buccal bony window area in the experimental and control groups respectively. Similarly, palatal window showed 99.03% and 100% reduction in the experimental and control groups respectively. No significant difference in both buccal and palatal window reduction was noticed between the groups. A total of 14 cases (seven in the experimental group and seven in the control group) showed complete closure of through-and-through bony window. No significant difference in clinical, 2D and 3D radiographic healing, percentage reduction in area and volume was observed between the experimental and control groups ($p > .05$). Neither the area nor the volume of lesion, and the size of buccal or palatal window had significant effect on healing of through-and-through defects.

Conclusion: Endodontic microsurgery results in high success rate in large periapical lesions with through-and-through communication with more than 80% reduction in

volume of lesion and size of both buccal and palatal window after 1 year. A mixture of type-1 collagen particles and i-PRF, adjunct to periapical micro-surgery did not improve the healing in through-and-through periapical defects.

KEYWORDS

cone beam computed tomography, endodontic microsurgery, guided tissue regeneration, injectable-platelet-rich fibrin, through-and-through periapical bone defect, type 1 collagen particles

INTRODUCTION

Peri-radicular lesion with loss of lingual/palatal and buccal cortex due to progression of pathology or creation of access during surgical treatment can result in 'through-and-through periapical defect' (TTPD; Von Arx & Cochran, 2001). Healing is significantly reduced following periapical surgery when both cortices are lost (Hirsch et al., 1979). During the healing phase, fibrous tissue can herniate into these defects on the buccal and palatal aspects limiting complete healing with bone regeneration (Baek & Kim, 2001; Dahlin et al., 1990). Large defect windows, particularly those that involve the buccal and palatal aspects of the periapical bone can provide a pathway for the microbial contamination and tissue ingrowth, negatively impacting complete healing and long-term outcomes. The closure of the cortical window may be considered a critical factor in the healing outcome of TTPD. These defects would require intervention with endodontic microsurgery (EMS) using regenerative material. The use of bone grafts and/or autologous platelet concentrates (APCs) may facilitate healing and regeneration of the affected tissues in TTPD.

Guided tissue regeneration (GTR) or guided bone regeneration techniques have been demonstrated in animal/histological studies to promote healing in TTPD (Baek & Kim, 2001; Dahlin et al., 1990; Murashima et al., 2002). Membranes and grafts act as space maintainer, allowing appropriate cells to repopulate the defect while also secluding the epithelial cells, promoting better regeneration (Bashutski & Wang, 2009). In contrast to experimental and animal studies, human studies have reported variable results. Taschieri et al. (2007) evaluated two-dimensional healing following the use of collagen membrane and anorganic bone graft in adjunct to periapical surgery in four-wall and through-and-through defects. They reported no significant benefit of GTR on overall pool of patients, with better outcomes observed in 4-wall defects independent of GTR, compared to through-and-through defects. On the other hand, Taschieri et al. (2008) using the same grafting techniques in TTPD, found significantly better radiographic healing in the GTR group. Only two clinical trials have evaluated 3D healing in TTPD after periapical surgery (Dhamija et al., 2020; Parmar et al., 2019). While Parmar et al. (2019) found no significant improvement following the use of the collagen membrane; Dhamija et al. (2020) used platelet-rich plasma (PRP) and found significantly greater success in the PRP group compared to control. Both studies observed more than 80% reduction in area and volume of lesion in the control and experimental groups.

Autologous platelet concentrates are blood products characterized by higher concentrations of platelets and leucocytes as compared to baseline (whole blood). The platelets release growth factors and cytokines from their alpha granules which mediate cell migration, proliferation and differentiation along with angiogenesis, and extracellular matrix synthesis, which are essential for tissue regeneration (O'Sullivan & Ríordáin, 2022). Periodontology, Oral Surgery, Esthetic and Implant Dentistry Organization has categorized APCs into four types based on their cell content (mostly leucocyte) and fibrin architecture. 'P-PRP' and 'L-PRP' have a low-density fibrin network and require the addition of anticoagulant and a two-step centrifugation process. On the other hand, 'P-PRF' and 'L-PRF' contain high-density fibrin network and can be prepared using a single-step centrifugation process without the need for anticoagulant (Ehrenfest et al., 2013). The presence of leucocytes is a key factor for neutralizing infectious pathogens (antibacterial action) and regulating immune reactions. Leucocytes may have an impact in the healing properties of APCs (Dohan et al., 2006; Kobayashi, Flückiger, et al., 2016; Kobayashi, Saita, & Nishio, 2016; Miron & Choukroun, 2017).

PRF formulations have distinct growth factor release kinetics. Its three-dimensional nanofibre structure allows for the steady release of growth factors for up to 10 days, compared to PRP where a significant amount of proteins are unleashed within the first hour (Kobayashi, Flückiger, et al., 2016; Kobayashi, Saita, & Nishio, 2016). PRF can function as a scaffold or resorbable membrane, supporting the delivery of cell lineages to the damaged areas (He et al., 2009; Masoudi et al., 2016). PRF variants are largely dense or solid gels that cannot be injected or mixed with other biomaterials used for bone regeneration. Higher centrifugation speed results in leucocytes predominantly found at the bottom of the PRF scaffolds (Ghanaati et al., 2014). Recently, advanced-PRF and injectable-PRF (i-PRF) have been introduced based on

low-speed centrifugation concept that favours more uniform distribution of leucocytes and significantly higher release of growth factors compared to traditional PRF (Fujioka-Kobayashi et al., 2017). i-PRF is a liquid concentrate that maintains its liquid form for about 10–15 min following centrifugation. Biomaterials can be added to this liquid as a combination strategy for delivery into host tissues (Miron & Zhang, 2018). Studies have shown that i-PRF promoted better cellular migration, proliferation and differentiation than PRP, and increased the expression of key proteins involved in tissue regeneration, such as TGF-β, PDGF and COL1a2 (Miron et al., 2017). Furthermore, a considerable elevation in expression of ALP, Runx2 and osteocalcin, along with positive immuno-fluorescent staining for osteocalcin, was observed in i-PRF compared to the PRP (Wang et al., 2018). The combination of i-PRF and bone substitute materials has been shown to enhance human osteoblast viability, proliferation, migration and attachment (Kyyak et al., 2020, 2021). However, despite these promising results in laboratory studies, clinical studies on i-PRF for bone regeneration are still lacking.

An ideal biomaterial for bone substitute should be physicochemically stable, biocompatible, non-toxic and biodegradable. One such biomaterial is the collagen-based materials derived from animal tissues and tendons (Etherington, 1977). Type-1 collagen is considered as the gold standard in tissue engineering, and the possible degradation by human collagenases is responsible for its widespread use in biomedical applications (Parenteau-Bareil et al., 2010; Rico-Llanos et al., 2021). Collagen-based products are available in the form of membrane, sponge/particles. It functions as a scaffold for new tissue growth and helps in attachment of the cells, thus promotes healing (Khan & Khan, 2013). Collagen has been used as graft material to promote regeneration of bone in extraction socket bone defects and sinus augmentation surgery (Gülsen & Dereci, 2019; Tsai et al., 2019). The efficacy of i-PRF in combination with type-1 collagen particles for promoting healing in adjunct to periapical surgery in TTPD has not been demonstrated.

The aim of the present study was to evaluate the effect of a mixture of i-PRF and type-1 collagen particles on the healing of through-and through periapical lesion and the closure of bony window following EMS.

MATERIALS AND METHODS

Study design

The study was designed as a parallel, double-blind randomized controlled trial and followed the guidelines outlined in the Preferred Reporting Items for Randomized Trials in Endodontics (PRIRATE) 2020, ensuring compliance with reporting standards (Nagendrababu et al., 2020). Approval to conduct the study was obtained from the Institutional Ethical Committee (PGIDS/IEC/2019/36) and the clinical trial was registered with ClinicalTrials.gov (NCT04391725). Enrolment for the study was done from December 2019 to June 2020 after obtaining written informed consent from all the participants.

Patient selection

Participants presenting with persistent clinical signs and symptoms in maxillary anterior teeth with large periapical lesion underwent periapical radiographic and CBCT examination as a part of eligibility assessment. The inclusion criteria were as follows:

- Consenting patients aged 16–45 years.
- Non-contributory medical history (American classification of anaesthesiologists [ASA] I and II).
- Maxillary anterior teeth with persistent clinical symptoms and large periapical lesion which had confirmed loss of palatal cortex with or without loss of buccal cortical plate on CBCT.

Exclusion criteria—Patients with systemic diseases and infectious complications, bleeding or clotting disorders, a history of medications such as platelet-function inhibitors (e.g. NSAIDs, acetylsalicylic acid, antiplatelet drugs), a history of chemotherapy or radiotherapy, a history of major traumatic injury, pregnant and lactating women and smokers. Additionally, periodontally compromised teeth with pocket depth greater than 6 mm, grade-3 mobility and unrestorable teeth with fractured or perforated roots were excluded from the study.

Sample size

The success rate of through and through periapical bone defects following periapical surgery was reported 25% (Hirsch et al., 1979). When APC (PRP) was used as GTR in the same defects, the success rate was reported to be 87.5% (Dhamija et al., 2020). With $\alpha = 5\%$ and a power of 90%, a minimum of 15 participants in each group is required using a method of comparing two proportions. Expecting around 20% attrition, a total of 38 patients (19 in each group) were included (Figure 1).

Randomization and blinding

The participants were randomly allocated to the experimental and control groups by using an automatic randomization method (www.random.org) after root end

filling. As per enrolment order, patients were given a sequential number by the co-investigator. Based on this number, the operator was informed about which treatment protocol would be applied (mixture of i-PRF and collagen or control). The patient and the operator were blinded throughout the procedure until the phlebotomy was performed for i-PRF preparation, after which blinding was no longer possible. Two calibrated and blinded evaluators, who were not involved in the study, assessed the healing outcome. In case of disagreements scoring outcomes or calculating measurements between the two examiners, the final value was recorded after consensus was achieved through discussion.

Radiographic acquisition

For all participants, standardized exposure parameters (70 kvp, 3.5 mAs, 0.2 s) and Rinn paralleling technique (Dentsply Rinn) with SIDEXIS XG sensor (Dentsply Sirona) were used for periapical radiographs at pre-operative and 12-month post-operative follow-up. Pre-operative and 12-month post-operative CBCT images were captured using CS 9300 3D machine (Carestream Dental LLC) with a field of view of 50 mm × 50 mm field of view and a voxel size of 0.09 mm. CS 3D imaging software (Carestream Dental) was used to analyse coronal, axial and sagittal planes of CBCT images. The images were viewed on a 15.6 inch monitor (HP i3 D8Q7C4V1) with a resolution of 1280*1024 pixels in a subdued lighting.

Outcome variables were assessed at 12-month follow-up that included, (i) Reduction in the buccal and palatal cortical bony window area and subsequent closure of through and through defect. (ii) 2D and 3D radiographic healing. (iii) Percentage reduction in area and volume of periapical lesion. (iv) Clinical outcome measures.

Cortical bony window area measurements

Pre-and post-operative DICOM files of 3D reconstructed images are transferred to RadiAnt (64 bit) software. The area (in px2) of the cortical bony window opening at buccal and palatal aspect was measured by two evaluators. Pre- and post- operative cortical bony window area measurements were compared (Figures 2 and 3 represent the pre- and post-operative cortical bony window area of buccal and palatal aspect in the i-PRF and control groups respectively).

2D and 3D radiographic healing assessment

2D healing was determined using 'Molven's criteria' (Molven et al., 1987), whereas modified PENN 3D criteria were used to assess 3D healing (Schloss et al., 2017).

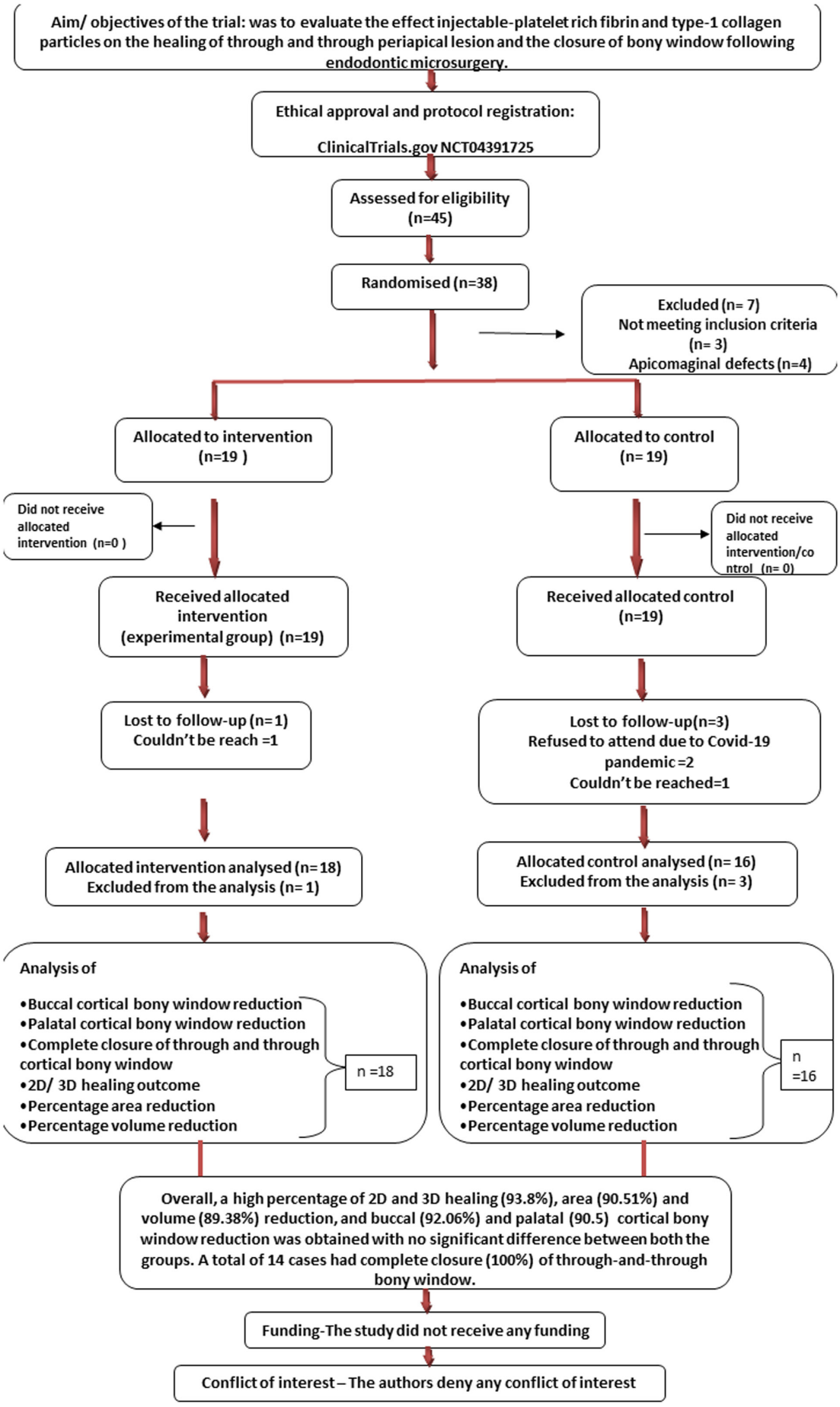
Molven's criteria for the assessment of 2D healing:

a. *Complete healing*: The periodontal space is restored to its normal width, but not more than twice the width along the resected surface or the lamina dura should be reconstituted around the apex. Not more than 1 mm^2 defects adjacent to the root filling in lamina dura. The structure and radio-opacity and of the regenerated bone may differ from that of adjacent healthy bone
b. *Incomplete healing*: Asymmetric radiolucency around the apex and connected to the periodontal space at an angle with evidence of bone healing with a decrease in size of radiolucency around its periphery.
c. *Uncertain healing*: The radiolucency size may have decreased, but remains more than twice the normal periodontal space with circular or semi-circular or funnel shape radiolucency symmetrically located around the apex.
d. *Unsatisfactory healing*: Radiolucency remains unchanged or has increased in size.

Modified PENN 3D criteria for the assessment of 3D healing are as follows

a. *Complete healing*: Periodontal space and lamina dura are restored to their normal width over the entire resected and unresected root surfaces. A slight widening of apical periodontal space but not exceeding two times the size of lamina dura of the adjacent healthy roots. It is acceptable for a small defect to be present in the lamina dura around the root-end filling. Complete repair of bone can be confirmed by the presence of a distinct hard tissue or lamina dura surrounding the surface of the resected root. Small radiolucency around the root end filling is acceptable.
b. *Limited healing*: Complete healing can be seen around the resected root surface, but the area may also demonstrate certain conditions that indicate limited healing. These conditions include interruption in continuity of cortical plate low-density area, an angular asymmetric area of relatively less density located apically, incomplete bone formation in the former osteotomy area, lack of bone coverage, or failure of periodontal space over areas with physiological fenestration or pre-existing periodontal disease

FIGURE 1 PRIRATE flowchart—the study design and flowchart of study participants throughout the trial.


Aim/ objectives of the trial: was to evaluate the effect injectable-platelet rich fibrin and type-1 collagen particles on the healing of through and through periapical lesion and the closure of bony window following endodontic microsurgery.
Ethical approval and protocol registration:
ClinicalTrials.gov NCT04391725
Assessed for eligibility (n=45)
Randomised (n=38)
Excluded (n= 7)
Not meeting inclusion criteria (n= 3)
Apicomaginal defects (n=4)
Allocated to intervention (n=19)
Allocated to control (n= 19)
Did not receive allocated intervention (n=0)
Did not receive allocated intervention/control (n= 0)
Received allocated intervention (experimental group) (n=19)
Received allocated control (n=19)
Lost to follow-up (n=1)
Couldn't be reach =1
Lost to follow-up(n=3)
Refused to attend due to Covid-19 pandemic =2
Couldn't be reached=1
Allocated intervention analysed (n=18)
Excluded from the analysis (n= 1)
Allocated control analysed (n= 16)
Excluded from the analysis (n= 3)
Analysis of
•Buccal cortical bony window reduction
•Palatal cortical bony window reduction
•Complete closure of through and through cortical bony window
•2D/ 3D healing outcome
•Percentage area reduction
•Percentage volume reduction
n =18
Analysis of
•Buccal cortical bony window reduction
•Palatal cortical bony window reduction
•Complete closure of through and through cortical bony window
•2D/ 3D healing outcome
•Percentage area reduction
•Percentage volume reduction
n =16
Overall, a high percentage of 2D and 3D healing (93.8%), area (90.51%) and volume (89.38%) reduction, and buccal (92.06%) and palatal (90.5) cortical bony window reduction was obtained with no significant difference between both the groups. A total of 14 cases had complete closure (100%) of through-and-through bony window.
Funding-The study did not receive any funding
Conflict of interest – The authors deny any conflict of interest

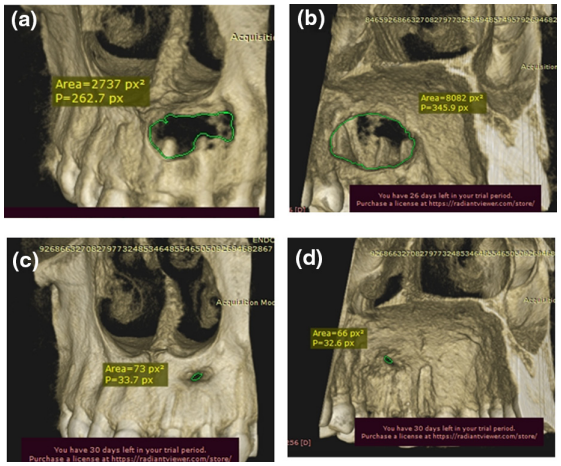


FIGURE 2 Experimental group (a) Pre-operative buccal cortical window area. (b) Pre-operative palatal cortical window area. (c) Post-operative buccal cortical window area. (d) Post-operative palatal cortical window area.

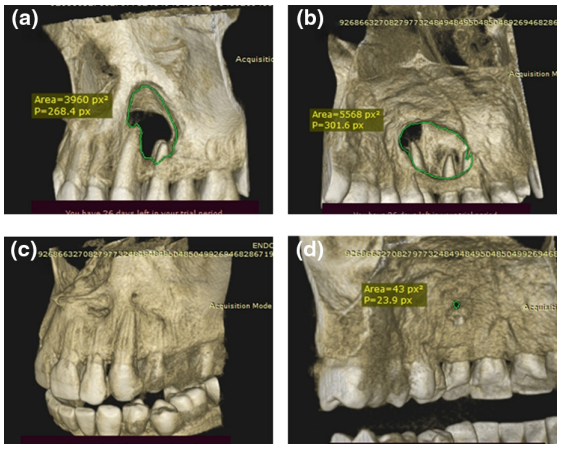


FIGURE 3 Control group (a) Pre-operative buccal cortical window area. (b) Pre-operative palatal cortical window area. (c) Post-operative buccal cortical window area. (d) Post-operative palatal cortical window area.

c. *Uncertain healing*: The volume of radiolucency appears to have decreased, with a thickness of periodontal space that is either more than twice the width or a symmetrically located, funnel-shaped extension of radiolucency of periodontal space around the apex of involved root.
d. *Unsatisfactory healing*: The volume of low-density area at apex appears unchanged or has increased.

The success of radiographic healing was determined based on two categories: complete and incomplete/limited healing. Failure of radiographic healing was defined by two other categories: uncertain and unsatisfactory outcomes.

Lesion area and volume assessment

Pre- and post-operative periapical lesion area and volumes were measured with Corel draw graphic suite X7 (64-bit) software and ITK- SNAP software (version 3.8.0) respectively (Dhamija et al., 2020; Parmar et al., 2019). To measure the lesion area, a periapical radiographic image was imported to Corel draw graphic suite X7 (64-bit) software, and the size of the defect was measured in square millimetres with the assistance of 1 × 1 mm grid superimposed on image (Figure 4). The volume of the periapical lesion was determined using ITK- SNAP software (version 3.8.0). The process involved transferring the DICOM data obtained to the software and using segmentation technique to measure the volume of lesion. The image was sliced at intervals of 0.09 mm, and the process involved three steps, namely—initialization, pre-segmentation and evolution. During initialization, periapical defect was outlined and selected in all three planes (axial, sagittal and coronal). In pre-segmentation, entire defects were repeatedly filled with automated spherical fillers and manual corrections ('bubbles') and the last step, evolution, generated the well-defined size of the defects that could be envisaged in three dimensions (Figure 5). After the process was completed, the software provided the exact volume of the defects in mm^3.

Clinical outcome measures included tooth survival, pain, swelling, tenderness to palpation or percussion, mobility, periodontal pocket (>3 mm), presence of sinus tract and satisfactory soft tissue healing which were assessed clinically at 1 week post-operatively, and further at 6 and 12 month follow-up.

Surgical procedure

A strict COVID-19 precaution protocol was followed by the operator throughout the study period. Surgery was performed wearing a personal protective equipment to prevent infection. The operatory room was fumigated prior to the surgical procedure. The surgical procedures for all the patients were performed by the post-graduate student (ACM) using a surgical operating microscope at 8× to 16× magnification (OPMI PICO; Carl Zeiss). Predictable anaesthesia and haemostasis were obtained at the surgical site using 2% lidocaine with 1:80 000 adrenaline. Following the administration of local anaesthetics, the initial incision was made and a full-thickness mucoperiosteal flap was elevated. An ostectomy was performed to gain access to the root-end. Periradicular curettage in conjunction with root-end resection, followed by 3 mm coaxial root-end preparation using reverse angle ultrasonic tips S127D (Satelec Corp). The prepared cavity

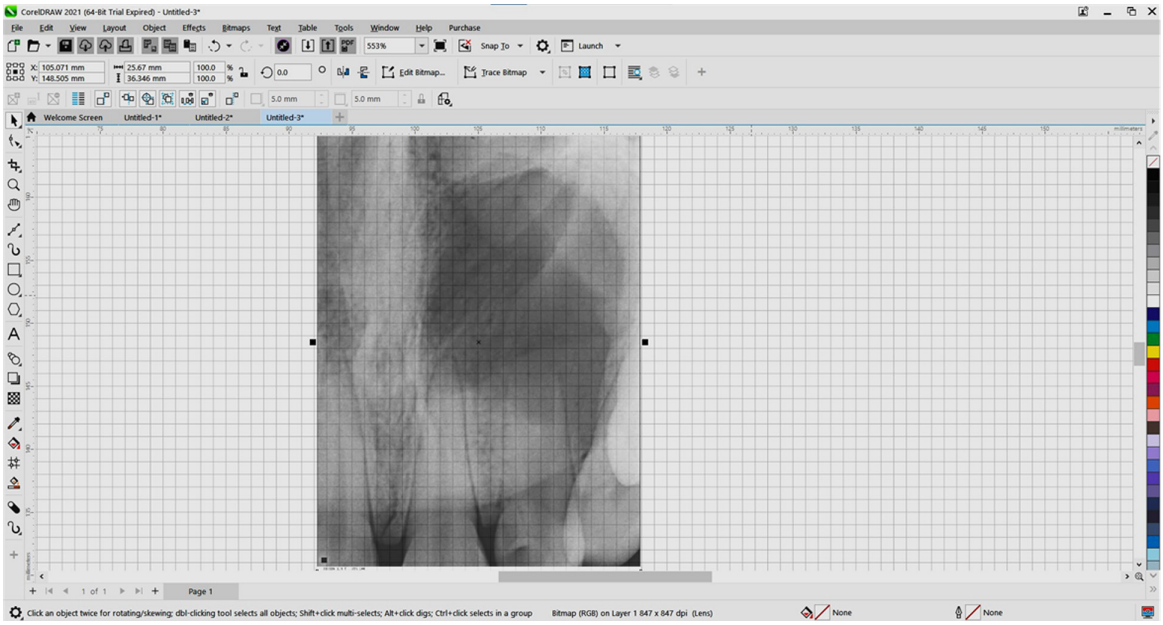


FIGURE 4 Image representing the measurement of area of the lesion using Corel draw graphic suite X7 (64-bit) software.

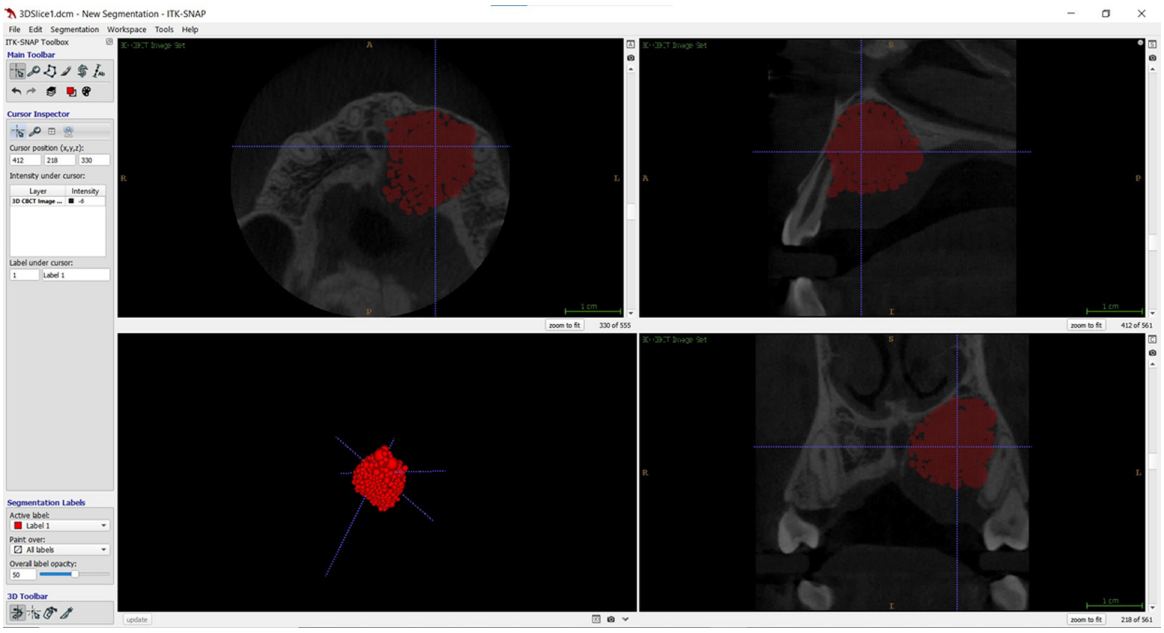


FIGURE 5 Image representing the measurement of volume of the lesion using ITK-SNAP software.

was rinsed with saline and dried, and sealed with MTA (Dentsply). All the steps, which involved addressing the root-end, were done under high magnification (14–26×; OPMI PICO; Carl Zeiss). Pathological tissue was sent for histological examination.

Experimental group

i-PRF was prepared according to protocol described by Ola M Ezzatt (2018). Eight millilitres of blood was collected in two plastic vacutainers (4 ml in each) without anticoagulant from antecubital vein. Centrifugation of the blood sample was performed immediately at 700 rpm for 3 min in a centrifugation machine with a rotator head (T-8M with rotor angulation of 33° and a radius of 88 mm). After completion of centrifugation, the upper yellow buffy coat (yellow i-PRF) and red zone (red i-PRF) were labelled as i-PRF (Figure 6). A 21- gauge needle was used to collect i-PRF, which was then mixed immediately without delay with type-1 collagen particles (Enzomac CM Particles, Macleods Pharmaceuticals Pvt Ltd) in a sterile container.

A mixture of i-PRF and collagen was placed in the defect (Figure 7d,e) within 10 min of i-PRF preparation, the flap was repositioned and suturing was done using 5-0 silk suture. Figure 7a-c,f–h represents pre-and post-operative 12-month CBCT images of the experimental group respectively.

Control group

After removal of pathological tissue, surgical defect was allowed to fill with blood (Figure 8d,e) and the flap was adapted and sutured (5-0 silk suture; Ethicon, Inc., Johnson & Johnson). Figure 8a–c,f–h represents pre-and post-operative 12-month CBCT images of the control group respectively.

All patients were advised by a resident doctor (K.G.) who was not involved in the study to follow post-operative instructions. An oral analgesic/anti-inflammatory drug, ibuprofen 600 mg for 3 days, was prescribed to the patients. Sutures were removed on the 5th post-operative day. At 12 months' follow-up, patients were recalled and assessed for clinical and radiographic healing.

Statistical analysis

The statistical analysis was performed using SPSS v.20 (SPSS/IBM). The Kolmogorov–Smirnov test for normality was used to assess the distribution of continuous data. Since data were not normally distributed non-parametric Mann–Whitney test was used to assess the quantitative variables and differences between the groups. Wilcoxon sign rank test was applied to determine the differences within groups (pre vs. post-12m; Table 1). Categorical variables were analysed using the Chi-square tests. Multiple linear regression analysis was applied to evaluate the association of pre-operative factors (pre-operative area and volume of the lesion, pre-operative buccal and palatal bony window area) on the dependent variables (percentage reduction in area and volume of the lesion, percentage reduction of the buccal and palatal window area) and Multiple logistic regression was applied to predict the value of dependent variable (2D and 3D healing outcome), complete closure of the bony window from the independent variables (pre-operative area and volume of the lesion, pre-operative buccal and palatal bony window area).

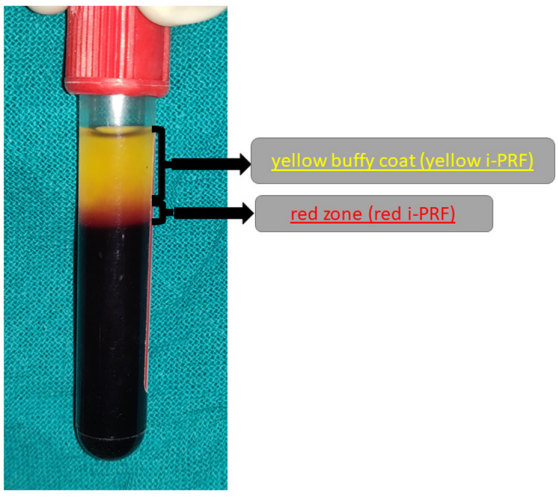


FIGURE 6 Image representing the zones of i-PRF used in the present study.

RESULTS

A total of 38 patients (19 in the experimental group and 19 in the control group), with 68 teeth (32 in the experimental group and 36 in the control group) were included in the study which was carried out from December 2019 to June 2020. Four patients were lost to follow-up (experimental group: $n=1$, control group: $n=3$). Two patients were unreachable and the other two patients declined to attend follow-up visit due to COVID-19 pandemic. Demographic distribution of the patients and pre-operative prognostic factor distribution are summarized in Table 2. No significant difference between the groups was observed in respect of age, gender, number of teeth involved, pre-operative pain and swelling, previous root canal treatment, intact buccal cortex, pre-operative area and volume of the lesion, area and maximum diameter of pre-operative buccal and palatal window (Table 2). At 12 months, 18 patients in the experimental group and 16 patients in the control group were available for the follow-up. None of the patients reported any signs or symptoms of failure. The success rate with respect to clinical outcome measures was 100% in both the groups. No significant difference in percentage reduction of buccal and palatal cortical window area was depicted between the experimental group and the control group. The percentage reduction of the buccal cortical bony window area was 96.9 (78.28–100)% and 97.96 (91.21–100)% for the experimental and control groups, respectively, with p value of .512. Similarly, the percentage reduction of the palatal cortical bony window area was 99.03 (88.59–100)% and 100 (87.99–100)% for the experimental and control groups, respectively, with a p value of .494 (Table 3). Complete closure of through-and-through bony window (buccal + palatal) was observed in comparable proportion of the cases in both experimental (7/18) and control groups (7/16), with no statistically significant difference between them ($p=.774$; Table 3). 2D % reduction was 94.33 (87.37–100)% and 93.47 (89.69–96.82)%, 3D % reduction was 95.17 (89.52–98.61)% and 91.98

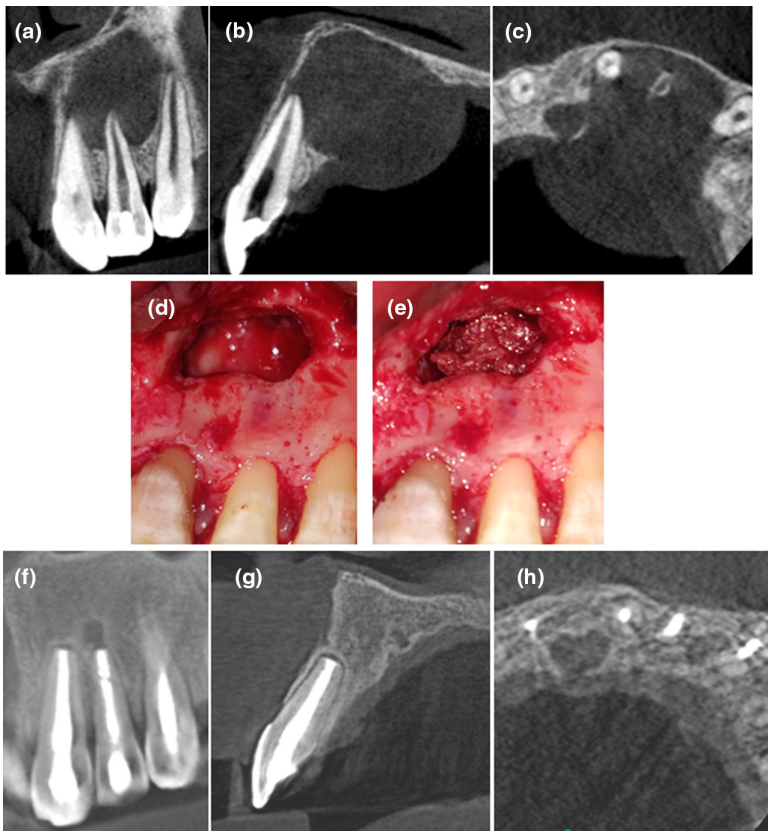


FIGURE 7 Experimental group (a) Pre-operative CBCT coronal view. (b) Pre-operative CBCT sagittal view of maxillary left central incisor. (c) Pre-operative CBCT axial view. (d) Bone defect after removal of pathological tissue. (e) i-PRF mixed with collagen particles and packed in the defect. (f) Post-operative CBCT coronal view. (g) Post-operative CBCT sagittal view of maxillary left central incisor. (h) Post-operative CBCT axial view.

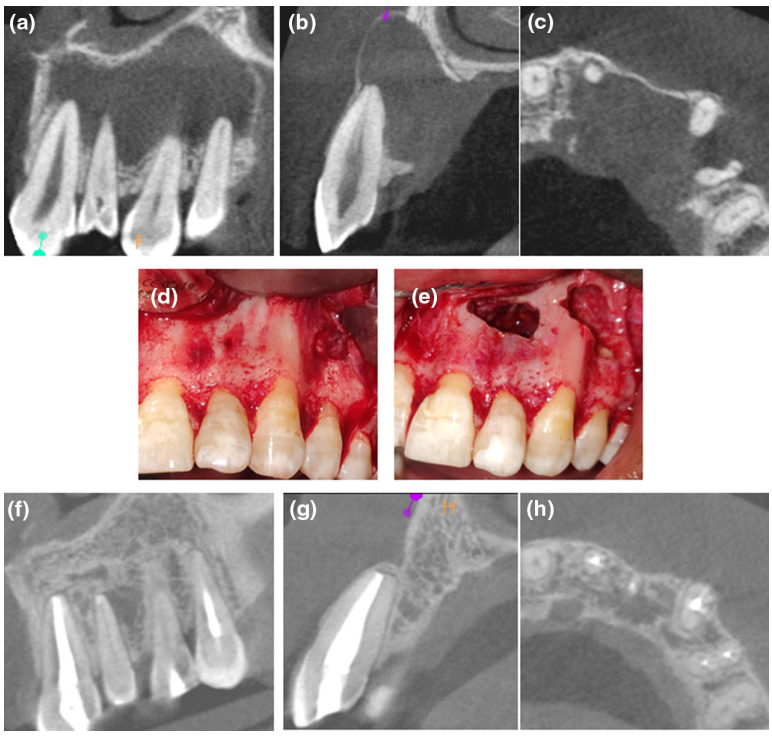


FIGURE 8 Control group (a) Pre-operative CBCT coronal view. (b) Pre-operative CBCT sagittal view of maxillary left canine. (c) Pre-operative CBCT axial view. (d) Flap elevation. (e) bone defect after removal of pathological tissue. (f) Post-operative CBCT coronal view. (g) Post-operative CBCT sagittal view of maxillary left canine. (h) Post-operative CBCT axial view.

(73.54–95.71)% in the experimental and control groups respectively. 2D and 3D percentage reduction between the groups did not have a significant difference (2D % reduction-$p = .472$ and 3D % reduction p value $= .167$; Table 3). According to modified PENN 3D scoring, five cases (three in the experimental group and three in the control group) achieved complete healing, while 27 cases (15 in the experimental group, 12 in the control group) were categorized as limited healing. Using Molven's criteria, a total of 19 cases (10 in the experimental group and nine in the control group) were classified as complete healing, and 13 cases (eight in the experimental group, five in the control

TABLE 1 Intra-group comparison (pre-op v/s post-op 12 months) of area and volume of the lesion, buccal and palatal cortical window area.

	Experimental group (18)		Control group (16)	
	Pre-op	Post-op 12 month	Pre-op	Post-op 12 month
	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)
Area of the lesion (mm^2)	132 (101.75–186.5)	6 (0–15.25)	141 (115.25–159)	11 (3.25–22.75)
p-value	<.001		<.001	
Volume of the lesion (mm^3)	1394.92 (613–1777.72)	44.68 (12.42–131.9)	883.25 (536.12–1619.50)	73 (33.07–151.8)
p-value	<.001		<.001	
Buccal cortical window area (px^2)	305 (144.75–2514.75)	48 (0–148.12)	1941.5 (683.50–3958.50)	36.5 (0–337.5)
p-value	<.001		<.001	
Palatal cortical window area (px^2)	2595.50 (1286.15–5363.37)	37.75 (0–344.5)	2448 (855.24–3423)	0 (0.00–0.00)
p-value	<.001		<.001	

group) showed incomplete healing. Two cases from the control group were considered to have uncertain healing based on both criteria. Successful healing was defined as achieving complete and limited/incomplete grades. The study showed 100% success rate for both 2D and 3D healing outcomes in the experimental groups and an 87.6% success rate (14/16) in the control group. Success rates for 2D and 3D healing were similar between the groups (Table 4). Fifteen samples in the experimental group and 12 samples in the control group were diagnosed as periapical granulomas, while histopathological reports confirmed three samples from the experimental group and four from the control group as inflammatory cysts. During post-operative follow-up, all respective lesions had successfully healed, except for two cases in the control group, which were identified as periapical granulomas and showed unsatisfactory 2D and 3D radiographic healing. Multiple linear regression analysis in this study revealed that pre-operative factors (area and volume of the lesion, and pre-operative buccal and palatal cortical window area) did not have a significant effect on the reduction of buccal or the palatal bony window. Multiple logistic regression analysis revealed no association of pre-operative predictive factors on 2D, 3D healing and on closure of bony window.

DISCUSSION

PRF has been suggested as a regenerative material in bone tissue engineering, either alone or in combination with other biomaterial (Farmani et al., 2021). However, the use of i-PRF with type-1 collagen in root-end surgery is a new approach that has not been studied in TTPD involving maxillary anterior teeth. In this study, we evaluated various measures of healing in TTPD at 1-year follow-up, with and without the composite matrix of i-PRF and type-1 collagen. These measures included patient-related outcomes, reduction of the buccal and palatal cortical bony window, reduction in the lesion size and volume, and, 2D and 3D radiographic healing.

The evaluation of cortical bony window reduction and closure is a useful indicator in the assessment of healing of TTPD. In the present study, similar percentage reduction in area of buccal and palatal cortical window was observed when measured using RadiAnt DICOM viewer software. The present results suggest that bone regeneration can occur in TTPD without the use of membranes or graft materials. EMS is preferable to extraction and implant placement as it promotes bone regeneration, crucial for long-term success. Large peri-radicular lesions causing pathological bony windows can be prevented with this treatment. Parmar et al. (2019) and Dhamija et al. (2020) using RACB index reported that complete reestablishment of the buccal cortical plate in 27% and 30% of the collagen membrane and control groups, and 21.4% and 41.9% of the control and PRP groups respectively (Dhamija et al., 2020; Parmar et al., 2019). Dhamija et al. (2020), reported faster regeneration of palatal cortical plate compared to buccal cortical plate. However, they have not reported about the percentage reduction of buccal and palatal cortical window area and closure of bony window.

The present study is in agreement with the result of Parmar et al. (2019), who showed no significant difference between the experimental and control groups, with both groups showing high success rate in 2D and 3D healing. However, these results contradict the findings of Dhamija et al. (2020), who reported a significantly lower success rate of 50% in the control group compared to PRP in 3D evaluation. According to modified PENN 3D criteria, complete and limited healing are considered to be successful, while uncertain and unsatisfactory healing are considered to be failure. The assessment of success utilizing modified

TABLE 2 Distribution of relevant pre-operative factors in experimental and control groups.

	Experimental (19)	Control (19)	*p*-value
Age (years)			
Median (Q1–Q3)	24.50 (21.50–31.25)	25 (24–29.75)	.339
Range	17–38	17–38	
Gender			
M	13 (68.4%)	12 (63.2%)	.732
F	6 (31.6%)	7 (36.8%)	
Tooth involved			
1 tooth	7 (36.8%)	5 (26.3%)	.485
>1 < 4 tooth	12 (63.2%)	14 (73.7%)	
Pre-operative pain			
Present	9 (47.4%)	11 (57.9%)	.516
Absent	10 (52.6%)	8 (42.1%)	
Pre-op swelling			
Present	12 (63.2%)	11 (57.9%)	.740
Absent	7 (36.8%)	8 (42.1%)	
Previous rct			
Present	1 (5.3%)	3 (15.8%)	.604
Absent	18 (94.7%)	16 (84.2%)	
Intact buccal cortex			
Intact	4 (21.1%)	1 (5.3%)	.340
Fenestration	15 (78.9%)	18 (94.7%)	
Area of the lesion (mm^2)			
Median (Q1–Q3)	132 (101.75–186.5)	141 (115.25–159)	.953
Range	39–303	54–281	
Volume of the lesion (mm^3)			
Median (Q1–Q3)	1394.92 (613–1777.72)	883.25 (536.12–1619.50)	.274
Range	89–4224.52	103.3–3003	
Pre-op buccal window area (px^2)			
Median (Q1–Q3)	305 (144.75–2514.75)	1941.5 (683.50–3958.50)	.072
Range	0–4804	0–8323	
Pre-op palatal window area (px^2)			
Median (Q1–Q3)	2595.50 (1286.15–5363.37)	2448 (855.24–3423)	.249
Range	296–8169	290–11 463	
Buccal window maximum diameter (mm)			
Median (Q1–Q3)	10.5 (4.87–16.60)	14.45 (7.43–26.4)	.154
Range	0–27	0–33.8	
Palatal window maximum diameter (mm)			
Median (Q1–Q3)	19.7 (13.77–25.05)	16.45 (10.52–23.17)	.953
Range	4.3–33.50	6.6–36.7	

PENN 3D carries an element of subjectivity, particularly in distinction of limited and uncertain healing, which may account for discrepancy between the current study and Dhamija et al. (2020). This study along with the previous reports also recorded reduction in area and volume of the lesion by over 80% in both the experimental and control groups (Dhamija et al., 2020; Parmar et al., 2019) and reiterates that the high success in TTPD can be attained with modern microsurgical concepts and hermetic seal of the root end with MTA. Unlike periodontal defects, periapical

TABLE 3 Inter-group comparison of percentage reduction in area and volume of the lesion, buccal and palatal window reduction percentage and complete closure of bony window (buccal + palatal).

	Experimental (18)	Control (16)	*p* value
Percentage area reduction (2D)			
Median (Q1–Q3)	94.33 (87.37–100) %	93.47 (89.69–96.82) %	.472
Volume percentage reduction (3D)			
Median (Q1–Q3)	95.17 (89.52–98.61) %	91.98 (73.54–95.71) %	.167
Percentage reduction of buccal window			
Median (Q1–Q3)	96.9 (78.28–100) %	97.96 (91.21–100) %	.512
Percentage reduction of palatal window			
Median (Q1–Q3)	99.03 (88.59–100) %	100 (87.99–100) %	.494
Complete closure of bony window (buccal + palatal)	7 (38.9%)	7 (43.8%)	.774
Incomplete/limited closure	11 (61.1%)	9 (56.3%)	

TABLE 4 2D and 3D success and failure evaluation in both treatment groups.

	Experimental (18) 2D/3D	Control (16) 2D/3D
Complete (1)	10/3	9/2
Incomplete/Limited (2)	8/15	5/12
Uncertain (3)	0/0	2/2
2D-and 3D-success (1 & 2)	18/18	14/14
2D-and 3D-failure (3 & 4)	0/0	2/2
p value	.369	.387

defects are closed defects and the marginal bone/the bone cuff and its overlying periosteum contribute to healing over time even without GTR material. The availability of surrounding marginal bone and the periosteum around them promoted the stimulation of progenitor cells to recruit in the defect area and their differentiation into dedicated cells results in the eventual healing of the periapical tissue (Lin et al., 2010).

In the present study, a combination of i-PRF and commercial collagen preparation Enzomac CM was used as a graft. Enzomac CM Particles also contain 2% w/w Mupirocin and 1% w/w, Metronidazole besides 90% w/w Collagen Peptide which might have also contributed in healing of surgical wound. In this study, i-PRF was prepared at [700 RPM (60 g) for 3 min]. Initially, i-PRF was introduced by Mourao et al. (2015), prepared at 3300 rpm for 2 min. However, most of the recent studies advocate 700 rpm centrifugation speed for 3 min for preparation of i-PRF. Reducing relative centrifugation force results in a significantly high number of leucocytes and platelet count and significantly higher release of growth factors and cytokines (Choukroun & Ghanaati, 2018; Miron et al., 2017; Wend et al., 2017).

After centrifugation, two fractions of the liquid concentrate were obtained: the yellow buffy coat or yellow i-PRF, and red zone or red i-PRF. It was observed that the yellow i-PRF had a dense fibrin network and exhibited better physical property; while the red i-PRF showed to have better biological properties by mobilizing the stem cells and promoting its proliferation. This is particularly beneficial for early-stage wound healing and regeneration of bone (Thanasrisuebwong et al., 2019, 2020). Therefore, in this study, we used yellow and red i-PRF in combination with collagen particles and manipulated them to obtain the benefits of both components.

Combination of i-PRF and collagen can be an alternative to expensive grafts for promoting healing after periapical surgery, as demonstrated by the present study. It is suggested to provide support to the overlying flap in large periapical lesions where there is loss of both buccal and palatal cortical plates. This combination is believed to have the potential to result in predictable healing outcomes. Gülsen and Dereci (2019), in a 3D evaluation, observed significantly more mesial and distal bone formation around implants 6 months after placement, when i-PRF and collagen plugs were used for sinus floor elevation ($p < .05$). In another retrospective clinical study, Amaral Valladao et al. (2020), observed that the bone grafts combined with i-PRF significantly increased bone thickness in patients with vertical and horizontal alveolar bone defects ($p < .001$ and $p < .005$ respectively). Although, the combination graft provided favourable healing outcomes at 1-year follow-up in the present study, the healing rates in the experimental group were not significantly different from the control group, where no graft was placed. The results are similar to those reported by Irdem et al. (2021), who did not find any significant effect of combination of demineralized

bovine bone matrix with liquid-PRF on new bone formation in maxillary sinus augmentation. Thus, a more comprehensive understanding of the effects of i-PRF and collagen in varied scenarios is required for their more judicious use.

Upon assessment of predictive factors such as pre-operative area and volume of the lesion, and pre-operative buccal and palatal cortical window area on healing outcome found no association on percentage reduction of buccal and palatal window, 2D and 3D healing, and closure of bony window. At 1 year post-RES, upon evaluating the predictability of these factors, found no significant effect on the outcomes of EMS of through and through periapical defects. Fewer studies found that age, gender, lesion size (>12 mm) and volume (>50 mm^3), perforation of the cortex, have negative predictive value on surgical healing outcomes (Song et al., 2013; Su et al., 2022). The results of the present study do not confirm these findings. These factors should be reassessed at long-term basis to evaluate the true predictability of the prognostic factors affecting healing (Pinto et al., 2020). A recent retrospective study, also found no significant effects of age, gender, pre-operative size of defect, tooth type, the presence of a post, the type of restoration and the apical extent of root filling on the outcome of EMS (Pallarés-Serrano et al., 2021).

As the study was conducted post pandemic, appropriate precautions were followed. All the patients underwent COVID-19 test prior surgery and were instructed to follow the COVID-19 norms. Neither patients nor the operator got infection throughout the study period. Following proper COVID-19 guidelines and protocol, transmission of infection was prevented.

The study has several limitations, including a small sample size and a short-term duration. Additionally, there was no histological evidence to confirm the regenerated tissue, and the through lesions were heterogeneous in nature. The measurements of the bony window area were recorded in 2D view of 3D reconstructed image, indicating the need of more reliable and consistent criteria to evaluate post-surgical healing outcomes using CBCT. To validate the findings of the present study, long-term follow-up with a large sample size is necessary. Future studies could focus on quantitative measurements such as density of bone formation. Furthermore, in future trials, Weasis software and MicroDICOM software could be employed as alternative options for image analysis, processing and complementing. Moreover, the current body of research on the role of APCs in soft tissue healing and their potential impact on improving post-operative quality of life is limited and requires further investigation (Del Fabbro et al., 2012).

CONCLUSION

Within the limitations of this study, it can be concluded that microsurgical approach has a high and predictable success in cases with through-and-through periapical bone defects. The use of injectable PRF with collagen did not result in significantly improved healing. Furthermore, pre-operative factors such as lesion size and loss of buccal and palatal cortical plate may not affect the healing outcome of EMS.

AUTHOR CONTRIBUTIONS

All the authors have contributed as per ICMJE criteria.

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This study has been registered at ClinicalTrials.gov with ID: NCT04391725.

CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ETHICS STATEMENT

As described in manuscript, the research was conducted following the approval from the Institutional Ethical Committee of Postgraduate Institute of Dental Sciences, Rohtak vide letter of PGIDS/IEC/2019/36.

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