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Clinical outcomes of regenerative endodontic treatment with injectable platelet-rich fibrin in mature necrotic teeth in a retrospective cohort study

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Research Article

Title: Clinical Outcomes of Regenerative Endodontic Treatment with Injectable Platelet-Rich Fibrin in Mature Necrotic Teeth in a Retrospective Cohort Study

Short Running Title: Regeneration in Mature Teeth

Keywords: Regenerative endodontic therapy, Pulp regeneration, Injectable platelet-rich fibrin, Mature permanent teeth

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

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This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Usak University Non-Interventional Clinical Research Ethics Committee (Decision No: 639-639-08, 29 April 2025). Written informed consent was obtained from all participants, as well as from the legal guardians of underage individuals.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Clinical Outcomes of Regenerative Endodontic Treatment with Injectable Platelet-Rich Fibrin in Mature Necrotic Teeth in a Retrospective Cohort Study

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ABSTRACT

Conventional root canal treatment eliminates infection in necrotic permanent teeth but does not restore the dentin–pulp complex. The clinical predictability of regenerative endodontic treatment in mature teeth remains uncertain. This study evaluated the clinical and radiographic outcomes of an injectable platelet-rich fibrin (i-PRF)-supported regenerative protocol in mature necrotic teeth. This retrospective two-center cohort study included mature permanent teeth presenting with pulp necrosis, closed apices, and periapical lesions with a periapical index (PAI) score ≥3 treated between 2022 and 2025 using a standardized i-PRF–supported regenerative endodontic treatment protocol. Periapical healing was assessed using PAI scores, with cone-beam computed tomography (CBCT) images serving as supplementary three-

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dimensional illustrations. Postoperative pain was recorded using the Visual Analog Scale (VAS). Statistical analysis was performed using the Wilcoxon signed-rank test, Friedman test with Dunn post hoc analysis, logistic regression, and receiver operating characteristic (ROC) curve analysis. A total of 27 teeth were included, with a mean follow-up of 22.5 months. Clinical success was achieved in 24 teeth (88.9%). Mean PAI scores decreased significantly from 4.3 ± 0.78 preoperatively to 1.07 ± 1.57 at follow-up ($p < 0.001$). CBCT images showed radiographic evidence of three-dimensional periapical healing. Postoperative pain was minimal, with median scores reaching zero within 72 hours and no need for analgesic medication. No significant predictors of treatment failure were identified. Within the limitations of this two-center retrospective study, i-PRF–supported regenerative endodontic treatment in mature necrotic teeth demonstrated favorable clinical outcomes, periapical healing, and low postoperative pain, suggesting its potential as a biologically based management approach.

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Keywords: *Injectable platelet-rich fibrin; Mature permanent teeth; Pulp regeneration; Regenerative endodontic treatment.*

INTRODUCTION

Conventional root canal treatment is the standard approach for teeth with necrotic pulp; however, it does not restore the biological integrity of the dentin–pulp complex and may result in loss of pulpal sensory function and altered tissue biology [1, 2]. Consequently, interest in biologically based regenerative endodontic treatment approaches has increased [3, 4].

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Regenerative endodontic treatment, a biologically based approach, aims to restore pulp-like tissue through the coordinated use of cell sources, biological signaling molecules, and supportive biomaterials [2, 5]. Cell-homing–based strategies aim to recruit endogenous mesenchymal stem cells via chemotactic signaling and depend on angiogenesis, cellular proliferation, and inflammation regulation [6]. In clinical practice, these processes have been supported using blood clot formation, platelet-rich plasma (PRP), platelet-rich fibrin (PRF), concentrated growth factor (CGF), and other biologically active scaffold materials [7-9].

The intracanal blood clot formed after induced bleeding contains multiple growth factors, including platelet-derived growth factor (PDGF), transforming growth factor–β (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and fibroblast growth factor (FGF); however, their low concentrations and short release duration may limit the effectiveness of regenerative endodontic treatment [10]. Accordingly, the American Association of Endodontists (AAE) recommends platelet-rich biological scaffolds, such as PRP, PRF, and autologous fibrin matrix (AFM), as alternatives to the conventional blood clot scaffold [4].

In the present study, injectable platelet-rich fibrin (i-PRF) was selected as the biological scaffold because its liquid form enables controlled and homogeneous intracanal distribution, unlike the gel-like consistency of PRP and PRF, which may complicate application in confined root canal anatomies [10]. Prepared using a low-speed centrifugation protocol, i-PRF forms a

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biologically active fibrin matrix rich in leukocytes and platelets, promoting close interaction with dentinal walls and supporting cell-homing–based regenerative processes [11, 12]. Its sustained growth factor release provides a favorable biological environment for tissue healing and may modulate early inflammatory responses, potentially influencing postoperative pain [10-14].

Although regenerative endodontic treatment have demonstrated high success rates in immature teeth [15, 16], outcomes reported for mature necrotic teeth remain heterogeneous. Current systematic reviews highlight significant methodological limitations within the existing literature and emphasize the lack of fully standardized regenerative endodontic treatment protocols [14, 17-20]. Furthermore, clinical evidence evaluating the success of regenerative endodontic treatment in mature teeth presenting with large periapical lesions remains limited [14, 16, 18, 19]. These limitations underscore the need for well-designed clinical studies using standardized regenerative endodontic treatment protocols in mature necrotic teeth.

This study aimed to evaluate the healing outcomes of i-PRF–supported regenerative endodontic treatment in mature necrotic teeth using a standardized two-center protocol. It was hypothesized that i-PRF application would be associated with enhanced periapical healing during follow-up and reduced early postoperative pain.

MATERIALS AND METHODS

Study Design

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This two-center retrospective cohort study was conducted in accordance with the PROBE 2023 guidelines and the Declaration of Helsinki, with ethical approval obtained from the Usak University Non-Interventional Clinical Research Ethics Committee (Decision No: 639-639-08; April 29, 2025). Ethical approval was granted specifically for the retrospective analysis of previously treated cases, and all data were analyzed in an anonymized manner.

Setting

Clinical and radiographic records of patients who underwent regenerative endodontic treatment between 2022 and 2025 at the Departments of Endodontics of Usak University and Ege University were retrospectively reviewed. All cases were treated using a standardized i-PRF–supported regenerative protocol applied uniformly at both centers, and only cases with complete clinical and radiographic documentation were included. Written informed consent for treatment and the use of clinical data for scientific purposes was obtained from all participants or their legal guardians.

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Sample Size

Given the retrospective design of the study, a post hoc sample size justification was performed using G*Power software (version 3.1.9.4) to assess whether the available sample size was sufficient to detect clinically meaningful effects. The analysis was based on a paired-samples t-test, with a two-tailed significance level of 0.05, an assumed effect size (dz) of 0.8 derived

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from previous clinical studies [14, 17] , and a statistical power of 0.85. The minimum required sample size was calculated as 17 teeth.

Eligibility Criteria

Eligibility criteria and case selection procedures are summarized in the flow diagram (Figure 1). Briefly, mature permanent teeth with necrotic pulp, a periapical index (PAI) score ≥3, and a minimum follow-up period of 12 months were included.

Regenerative Endodontic Treatment Protocol

All treatments were performed in two visits in accordance with the current American Association of Endodontists (AAE) guidelines [4]. At the first visit, access cavities were prepared under rubber dam isolation following local anesthesia without vasoconstrictor.

Root canal instrumentation was performed using hand K-files (Dentsply Maillefer, Ballaigues, Switzerland). Apical patency was initially confirmed using a size #10 K-file, and working length was established. In single-rooted teeth, canal preparation was carried out using a step-back technique. The master apical file (MAF) was determined based on the first file to bind at working length, and apical preparation was completed up to an approximate size of #40.

In multi-rooted teeth, apical patency was initially confirmed using a size #10 K-file, and working length was established. Canal negotiation and glide path preparation were performed using hand K-files, and final canal shaping was completed using a reciprocating file system (Reciproc R40, VDW, Munich,

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Germany) in accordance with the manufacturer's instructions. During root canal instrumentation, the apical foramen in mature teeth was progressively enlarged up to a size #40 K-file as part of the mechanical preparation. This step was performed to facilitate tissue ingrowth [21, 22]. Irrigation was carried out sequentially using 20 mL of 1.25% sodium hypochlorite (NaOCl) (CanalPro NaOCl, COLTENE, Altstätten, Switzerland), 5 mL of sterile saline solution, and 20 mL of 17% ethylenediaminetetraacetic acid (EDTA) (CanalPro EDTA 17%, COLTENE, Altstätten, Switzerland), delivered using a 30-gauge side-vented irrigation needle positioned approximately 2 mm short of the working length. Each irrigant was activated by passive ultrasonic irrigation (PUI) using an ultrasonic device (DTE, Guilin Woodpecker Medical Instrument Co., Guilin, China) with an irrigation activation tip, applied 2 mm short of the working length in three cycles of 20 seconds. After canal drying, calcium hydroxide ($Ca(OH)_2$) (Kalsin calcium hydroxide, Turkey) was placed as an intracanal medicament without extension into the apical third, and the access cavity was temporarily sealed using a glass ionomer cement (CIS) (Nova Glass F, IMICRYL, Konya, Turkey).

At the second visit, local infiltration anesthesia without epinephrine was administered under rubber dam isolation. $Ca(OH)_2$ was removed using 20 mL of 17% EDTA with ultrasonic activation for 5 minutes, followed by irrigation with sterile saline solution. Apical patency was reconfirmed using a size #10 K-file, and the working length was re-established prior to bleeding induction. Controlled apical bleeding was induced by gently extending a sterile #35 K-

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file approximately 1–2 mm beyond the apical foramen to allow initial clot formation. In cases where bleeding was not readily achieved, a size #25 K-file was advanced further apically. The tip of the #25 K-file was slightly pre-curved and extended beyond the apex to contact the surrounding periapical bone, thereby facilitating bleeding induction [4]. (Figure 2c). This step was performed with caution, taking canal anatomy into consideration.

Adequate bleeding was successfully achieved in all cases included in the study, and no case required exclusion due to inability to induce apical bleeding. Bleeding was typically observed shortly after apical stimulation; however, in some cases, it required up to a few minutes. The induced bleeding was sufficient to form a stable intracanal scaffold.

The same instrumentation, irrigation, and activation protocol was consistently applied to all canals in all teeth.

i-PRF Preparation and Placement of Cervical Barrier

For use as a biological scaffold, i-PRF was prepared by collecting approximately 9 mL of venous blood into a 9 mL additive-free vacuum tube (Vacusera, İzmir, Türkiye) and centrifuged at 700 rpm for 3 minutes at room temperature (Duo Centrifuge, Process for PRF, Nice, France), which corresponds to approximately 60×g relative centrifugal force (RCF), calculated based on the reported centrifuge rotor configuration [23] (fixed-angle rotor, ~40° angulation, radius 77–110 mm). This protocol was applied in accordance with the low-speed centrifugation concept to preserve cellular components and growth factor content. The platelet- and leukocyte-rich

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liquid fraction was carefully aspirated from the upper layer of the tube without disturbing the erythrocyte layer and gently injected into the canal space using a 30-gauge syringe, allowing complete filling of the canal up to the cemento-enamel junction. [11, 24]. Slight displacement of the initially formed blood clot was occasionally observed during i-PRF placement; however, this did not compromise scaffold stability (Figure 2a–d). A stabilization period of 10–15 minutes was allowed to facilitate fibrin network formation and in situ scaffold maturation, ensuring adequate resistance to displacement. Once scaffold stability was clinically confirmed, NeoPutty mineral trioxide aggregate (Avalon Biomed, USA) was placed in the cervical third at a thickness of 3–4 mm, followed by a CIS base. The coronal restoration was completed using a universal resin composite (Filtek Z350 XT, 3M, St. Paul, MN, USA), and a postoperative periapical radiograph was obtained.

Radiographic Assessment and Follow-up Imaging

Radiographic evaluation was performed using standardized periapical radiographs obtained with the parallel technique, supplemented by cone-beam computed tomography (CBCT) acquired preoperatively and at the 12-month follow-up. Periapical healing was assessed using the Ørstavik Periapical Index (PAI) [25], which was applied exclusively to periapical radiographs, while CBCT images were used to provide supplementary three-dimensional visualization of periapical healing.

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All images were evaluated independently and in a blinded manner by two experienced endodontists. Interobserver reliability was assessed in a pilot sample of 10 teeth and demonstrated excellent agreement (Cohen's kappa = 0.92). In cases of disagreement, a consensus score was determined by a third examiner.

Clinical Assessment and Postoperative Pain Recording

Clinical evaluation was performed at each follow-up visit and included assessment of spontaneous pain, percussion sensitivity, tenderness on palpation, sinus tract presence, and functional discomfort. Patients recorded daily postoperative pain levels using the Visual Analog Scale (VAS) during the first 7 days after treatment, and any analgesic use was documented for analysis. Patients were instructed to take analgesics only if necessary and to record the type and timing of any analgesic intake together with their VAS scores.

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Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics software (version 23.0; IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test, and nonparametric tests were applied as appropriate. Changes in pre- and post-treatment PAI scores were analyzed using the Wilcoxon signed-rank test. Postoperative VAS scores across time points were compared using the Friedman test with Dunn's post hoc analysis. In addition to time-based comparisons, postoperative pain was further characterized using absolute postoperative VAS values, peak postoperative

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pain (defined as the maximum VAS score recorded per patient), and the proportion of patients with VAS ≥4, consistent with previously established pain intensity categorizations [26]. Peak postoperative pain was summarized using the median and interquartile range (IQR), together with the observed minimum and maximum values. Logistic regression analysis was performed to identify potential factors associated with treatment failure. Univariate logistic regression analyses were performed prior to multivariable analysis to explore the association between each independent variable and treatment outcome. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive value of PAI and VAS scores. Categorical variables were expressed as percentages, and continuous variables as mean ± standard deviation or median (minimum–maximum). Statistical significance was set at $p < 0.05$.

RESULTS

A total of 27 teeth were included in the study. The patient cohort comprised 37% males and 63% females, with a mean age of 25.7 ± 9.89 years and a mean follow-up duration of 22.52 ± 5.52 months. Maxillary central and lateral incisors (teeth #11 and #12) were the most frequently treated teeth, each accounting for 22.2% of the sample. Overall, 81.5% of the teeth were single-rooted and 18.5% were multirooted; 66.7% were located in the maxilla and 33.3% in the mandible (Table 1).

At the final follow-up, clinical success was achieved in 24 of 27 teeth (88.9%), whereas 3 teeth (11.1%) were classified as unsuccessful. In unsuccessful

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cases, no radiographic evidence of healing or significant reduction in periapical lesion size was observed at the 12-month follow-up, and percussion sensitivity and functional discomfort persisted. These cases were therefore classified as failures at the 12-month evaluation. In contrast, all successful cases demonstrated complete resolution of percussion and palpation sensitivity, with no sinus tract formation or spontaneous pain. Following patient information and consent, conventional root canal treatment was subsequently performed in the unsuccessful cases. These cases were followed for clinical purposes but were excluded from further regenerative outcome analysis.

The mean PAI score decreased significantly from 4.3 ± 0.78 preoperatively to 1.07 ± 1.57 at the final follow-up, with a median reduction from 4 to 1 ($p < 0.001$) (Table 2). Representative periapical radiographs and CBCT images demonstrating three-dimensional periapical healing are shown in Figure 3.

Postoperative pain levels remained low throughout the observation period. Mean VAS scores increased slightly from 0.7 ± 1.07 preoperatively to 0.89 ± 0.93 at 6 hours, followed by a progressive decrease to 0.44 ± 0.58 at 12 hours, 0.37 ± 0.56 at 24 hours, 0.22 ± 0.51 at 72 hours, and 0.19 ± 0.48 on day 7. The Friedman test demonstrated a statistically significant difference in VAS scores across time points ($p < 0.001$). Dunn post hoc analysis revealed a significant difference only between the 6-hour and 7-day postoperative measurements, with no significant differences among the remaining intervals (Table 2).

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The median change in VAS score (ΔVAS) between baseline and day 7 was 0 (minimum −2, maximum 3). Temporal changes in VAS scores are illustrated in Figure 4. Although a transient increase in median VAS score was observed at 6 hours, VAS values declined from 12 hours onward and remained at 0 in most cases at 72 hours and at day 7.

No patient reported VAS scores ≥4 at any postoperative time point. Peak postoperative pain, defined as the maximum VAS score recorded per patient, had a median of 1 (range: 0–3). No postoperative analgesic intake was documented.

Univariate and multivariable logistic regression analyses did not identify any independent predictors of clinical failure ($p > 0.05$), and no significant associations were found between sex, age, jaw location, number of roots, and preoperative PAI and VAS scores and treatment outcome (Table 3). Because all multirooted teeth were classified as successful, this variable was excluded from the multivariable model due to categorical insufficiency.

ROC curve analyses evaluating the predictive value of PAI and VAS variables for treatment failure yielded no statistically significant area under the curve (AUC) values ($p > 0.05$). The AUC values were 0.542 for preoperative PAI, 0.292 for preoperative VAS, 0.417 for VAS at 6 hours, 0.625 for VAS at day 7, and 0.194 for ΔVAS (Table 4).

DISCUSSION

In this two-center retrospective clinical study, an i-PRF–supported regenerative endodontic treatment applied to 27 mature necrotic teeth,

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predominantly presenting with preoperative PAI scores of 4 to 5, achieved a clinical success rate of 88.9%. Treatment was associated with a significant reduction in mean PAI scores from 4.3 to 1.07, and comparative CBCT images showed radiographic signs of three-dimensional periapical healing over a mean follow-up of 22.5 months. Postoperative pain levels were minimal, with mean VAS scores remaining below 1 and median values reaching 0 from 72 hours onward, and no patient required nonsteroidal anti-inflammatory drug use. Taken together, these findings suggest that the i-PRF–supported protocol was associated with favorable radiographic healing and low postoperative discomfort in selected mature necrotic teeth.

Early clinical studies on regenerative endodontic treatment in mature necrotic teeth reported substantial heterogeneity in outcomes, with success rates ranging from 50% to 78% between 2010 and 2020 [16, 18, 19, 27]. This variability was primarily attributed to biological and anatomical constraints of mature teeth, such as a narrow apical foramen, limited regenerative cell migration, and instability of the intracanal blood clot, which were thought to reduce treatment predictability compared with immature teeth [16, 18]. In contrast, studies published after 2020 have reported markedly higher success rates, often approaching 80%–90%, which have been linked to greater protocol standardization, updated irrigation and intracanal medicament guidelines, and the use of biologically supportive scaffold materials [17-19, 27, 28]. The 88.9% clinical success rate observed in the present study falls within the range of more recent reports describing favorable healing

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outcomes in mature teeth, although differences in study design, case selection, and protocol characteristics should be considered when making direct comparisons.

Nevertheless, it should be recognized that the current evidence regarding regenerative endodontic treatment in mature teeth remains heterogeneous, and considerable variation exists in clinical practice. Multinational survey studies have reported substantial differences among endodontists in case selection, indications, and protocol implementation [29, 30]. These findings reflect ongoing uncertainty regarding the predictability of regenerative endodontic treatment in mature teeth. In this context, the present findings add clinical data derived from a standardized protocol in mature necrotic teeth.

The favorable clinical outcomes observed in the present study are likely multifactorial and should not be attributed to a single component of the treatment protocol. Both the biological characteristics of i-PRF and the standardized clinical approach applied may have contributed to the observed healing patterns. The sustained release of growth factors from i-PRF, together with its injectable consistency that allows adaptation to canal walls, may support a microenvironment conducive to tissue repair. However, protocol standardization, including controlled irrigation, intracanal calcium hydroxide medication, scaffold placement, and cervical barrier application, may have played an equally important role.

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A wide range of biological support materials has been reported in regenerative endodontic treatment studies, including autologous platelet concentrates such as blood clot, PRP, PRF and its various formulations, platelet-poor plasma (PPP), and CGF, as well as synthetic collagen- or hydroxyapatite-based scaffolds. Cell-based regenerative strategies using umbilical cord–derived mesenchymal stem cells or minced pulp tissue have also been described [7-10, 31, 32]. Despite methodological differences, these approaches share a common goal of establishing a biologically active intracanal microenvironment that promotes cell migration, angiogenesis, and tissue healing through sustained growth factor release within a three-dimensional fibrin network, providing a biological alternative to conventional obturation techniques [7-9, 15, 19].

Within this spectrum of biological support materials, i-PRF represents a leukocyte- and growth factor–rich liquid scaffold well suited for cell-homing–based regenerative approaches in mature teeth [10-13]. Compared with conventional blood clot scaffolds, platelet-rich plasma, and gel-type PRF membranes, i-PRF offers practical advantages related to its injectable consistency, high leukocyte content, and dense fibrin network formed through low-speed centrifugation [10-13]. These characteristics may enhance homogeneous adaptation and biological interaction within complex root canal anatomies, which is particularly relevant in mature teeth with narrow apical foramina and extensive periapical lesions [10-13, 15, 33]. i-PRF can be prepared using a rapid, cost-effective protocol without anticoagulants,

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enabling practical clinical use while preserving biological activity [10-12, 15, 32].

Although early studies reported lower predictability of regenerative endodontic treatment in mature teeth compared with immature teeth [15, 16, 18, 19], more recent evidence has demonstrated that substantial radiographic healing can be achieved in mature root canal systems [17, 18, 27, 31, 32]. In the present study, the significant reduction in mean PAI scores from 4.3 to 1.07 indicates effective periapical healing following the i-PRF–supported regenerative endodontic treatment protocol. Importantly, healing was assessed using both two-dimensional radiography and three-dimensional CBCT over a mean follow-up of 22.5 months, and was found to be compatible with stable volumetric bone fill rather than transient inflammatory resolution. This finding is consistent with experimental evidence showing that i-PRF maintains odontogenic differentiation and mineralization capacity even under inflammatory conditions, supporting tissue repair rather than mere resolution of inflammation [13].

Comparative CBCT images showed radiographic signs of reduction in lesion extent even in cases with cortical plate perforation or advanced periapical bone destruction. These observations suggest that the i-PRF–supported protocol was associated with radiographic evidence of osseous healing in mature teeth presenting with extensive lesions [15, 18, 19, 27]. These findings are consistent with the three-dimensional lesion regression reported in a randomized controlled trial using i-PRF [32], and with the bone

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remodeling described in resorptive cases treated with i-PRF [33]. Collectively, these observations support the concept that i-PRF may promote a biologically favorable microenvironment capable of facilitating healing in mature root canal systems with compromised or resorptive anatomy [11-13, 32, 33].

Postoperative pain levels in the present study were minimal, with mean VAS scores remaining below 1, median values declining to 0 by 72 hours, and no requirement for nonsteroidal anti-inflammatory drug use. These findings are consistent with previous clinical studies on regenerative endodontic treatments in both mature and immature teeth, which have reported that postoperative pain is generally mild and short-lived [14, 28, 32].

The low postoperative pain levels observed may be partly explained by the biological properties of i-PRF. Its leukocyte-rich fraction, obtained through low-speed centrifugation, enables sustained release of anti-inflammatory cytokines, particularly interleukin-10 (IL-10) and transforming growth factor–β1 (TGF-β1), with lower levels of interleukin-4 (IL-4), thereby modulating the inflammatory microenvironment and potentially reducing early postoperative edema and neural sensitization [10-12]. Under lipopolysaccharide-induced inflammatory conditions simulating pulpitis, i-PRF significantly reduces tumor necrosis factor-α and interleukin-1β expression and inhibits nuclear translocation of nuclear factor-κB in dental pulp cells [13]. This modulation of inflammatory signaling may represent a plausible biological explanation for reduced neural sensitization and may contribute to the minimal

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postoperative discomfort observed in the present study. However, it should be noted that inflammatory cytokine levels were not directly measured in this study, and this interpretation is supported by previously published experimental and biological studies demonstrating the anti-inflammatory properties of i-PRF [11-13]. Future studies incorporating biomarker analysis are required to clarify the biological mechanisms underlying this clinical observation.

Beyond the biological effects of i-PRF, operative factors, including controlled irrigation volumes, limited ultrasonic activation of NaOCl, and short-term $Ca(OH)_2$ application, may also contribute to reduced postoperative pain [14, 20, 28]. Collectively, these findings indicate that the i-PRF–supported regenerative endodontic treatment protocol represents a low-symptom clinical approach that enhances patient comfort in mature necrotic teeth.

The lack of significant clinical or demographic predictors of treatment failure in the logistic regression and ROC/AUC analyses should be interpreted cautiously, given the limited number of unsuccessful cases (n = 3). However, these findings are consistent with reports indicating that outcomes of regenerative endodontic treatment in mature necrotic teeth are not adequately explained by conventional clinical variables[18, 19, 27]. AUC values ranging from 0.19 to 0.62 further indicate that preoperative lesion severity, symptom intensity, and early postoperative pain changes have limited discriminatory value for predicting treatment failure.

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Systematic reviews indicate that commonly assessed clinical variables, including preoperative PAI score, root number, jaw location, and patient age, do not consistently predict treatment success. Instead, healing outcomes in mature teeth appear to depend on complex biological factors related to host response and the local microenvironment, supporting a multifactorial, host biology–dependent healing process [18, 19, 27, 32].

This two-center retrospective study is strengthened by the inclusion of advanced periapical lesions (PAI 4–5), a long mean follow-up of 22.5 months confirmed by radiographs and CBCT, and the use of a single standardized regenerative endodontic treatment protocol, enhancing internal validity and outcome reliability.

The feasibility of retreatment in unsuccessful cases suggests that this protocol may be cautiously considered in selected mature necrotic teeth, with the possibility of transitioning to conventional treatment when indicated.

This study has limitations, including its retrospective design, a limited number of unsuccessful cases affecting predictive power, and the use of a single i-PRF–supported protocol, which prevented comparison with alternative regenerative endodontic treatment approaches. Although standardized inclusion criteria were applied, the retrospective design may still influence patient selection through clinical decision-making processes, which may limit the generalizability of the findings.

Despite protocol standardization, operator-related variability cannot be fully excluded. Furthermore, although CBCT images were used to provide

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supplementary three-dimensional visualization of periapical healing, the multicenter design of the study involved CBCT datasets acquired using different devices and imaging parameters across the two centers, which precluded standardized quantitative volumetric analysis. Therefore, outcome assessment was based primarily on validated radiographic criteria, and this limitation should be considered when interpreting the findings.

Given the limited number of failure events, the results of the regression analyses should be interpreted cautiously and considered exploratory rather than definitive.

Future research should focus on larger prospective and comparative studies incorporating different biological scaffolds and objective biological predictors. In addition, the use of standardized CBCT protocols and quantitative volumetric analysis may provide a more objective assessment of periapical healing and improve the comparability of outcomes across studies. These approaches may help to better define the long-term effectiveness and clinical indications of i-PRF–supported regenerative treatments.

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CONCLUSION

This study shows that biologically mediated, cell-homing–based repair can result in clinically relevant and radiographically confirmed periapical healing in mature necrotic teeth. The i-PRF–supported regenerative protocol demonstrated high clinical success, minimal postoperative discomfort, and CBCT-confirmed three-dimensional periapical healing, even in teeth presenting with extensive lesions (PAI 4–5).

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These findings suggest that the limitations associated with apical closure in mature teeth may be partially overcome, and that this biologically based treatment approach, as associated with periapical healing, may represent a potential alternative to conventional obturation in carefully selected cases.

AUTHOR CONTRIBUTIONS

ECC: Conceptualisation, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review and editing, Supervision, Project administration. **TT**: Conceptualisation, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review and editing. **BY**: Investigation, Formal analysis, **MK**: Investigation, Formal analysis, **BC**: Visualisation, Writing – review and editing. **OT**: Writing – review and editing.

ETHICAL STATEMENT

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Usak University Non-Interventional Clinical Research Ethics Committee (Decision No: 639-639-08, 29 April 2025). Written informed consent was obtained from all participants, as well as from the legal guardians of underage individuals.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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FUNDING INFORMATION

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CLINICAL TRIAL NUMBER

Not applicable.

ACKNOWLEDGMENTS

The authors declare that there are no acknowledgments.

References

1. Murray, P. E., Garcia-Godoy, F. & Hargreaves, K. M. Regenerative endodontics: a review of current status and a call for action. *J. Endod.* **33**, 377–390. https://doi.org/10.1016/j.joen.2006.09.013 (2007).
2. Diogenes, A., Henry, M. A., Teixeira, F. B. & Hargreaves, K. M. An update on clinical regenerative endodontics. *Endod. Topics* **28**, 2–23. https://doi.org/10.1111/etp.12040 (2013).
3. Kim, S., Malek, M., Sigurdsson, A., Lin, L. & Kahler, B. Regenerative endodontics: a comprehensive review. *Int. Endod. J.* **51**, 1367–1388. https://doi.org/10.1111/iej.12954 (2018).
4. American Association of Endodontists. *AAE clinical considerations for a regenerative procedure* (American Association of Endodontists, Chicago, 2022).
5. Chrepa, V., Henry, M., Daniel, B. & Diogenes, A. Delivery of apical mesenchymal stem cells into root canals of mature teeth. *J. Dent. Res.* **94**, 1653–1659. https://doi.org/10.1177/0022034515596527 (2015).
6. Zhou, J., Su, Y. & Wang, S.-L. Progress and perspectives on cell-free regenerative endodontic therapy. *West China J. Stomatol.* **40**, 1. https://doi.org/10.7518/hxkq.2022.01.001 (2022).
7. Liu, H. et al. Biomaterial scaffolds for clinical procedures in endodontic regeneration. *Bioact. Mater.* **12**, 257–277. https://doi.org/10.1016/j.bioactmat.2021.10.008 (2022).
8. Darwish, O. B., Aziz, S. M. A. & Sadek, H. S. Healing potentiality of blood clot, S-PRF and A-PRF as scaffold in treatment of non-vital mature single rooted teeth with chronic peri-apical periodontitis following regenerative endodontic therapy: randomized clinical trial. *BMC Oral Health* **25**, 50. https://doi.org/10.1186/s12903-024-05378-0 (2025).

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9. Wei, X. et al. Expert consensus on regenerative endodontic procedures. *Int. J. Oral Sci.* **14**, 55. https://doi.org/10.1038/s41368-022-00206-z (2022).
10. Farshidfar, N. et al. Platelet-rich plasma (PRP) versus injectable platelet-rich fibrin (i-PRF): a systematic review across all fields of medicine. *Periodontol. 2000* https://doi.org/10.1111/prd.12626 (2025).
11. Miron, R. J. et al. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? *Clin. Oral Investig.* **21**, 2619–2627. https://doi.org/10.1007/s00784-017-2063-9 (2017).
12. Miron, R. J., Gruber, R., Farshidfar, N., Sculean, A. & Zhang, Y. Ten years of injectable platelet-rich fibrin. *Periodontol. 2000* **94**, 92–113. https://doi.org/10.1111/prd.12538 (2024).
13. Chai, J., Jin, R., Yuan, G., Kanter, V., Miron, R. J. & Zhang, Y. Effect of liquid platelet-rich fibrin and platelet-rich plasma on the regenerative potential of dental pulp cells cultured under inflammatory conditions: a comparative analysis. *J. Endod.* **45**, 1000–1008. https://doi.org/10.1016/j.joen.2019.04.002 (2019).
14. Ahmed, Y. E., Ahmed, G. M. & Ghoneim, A. G. Evaluation of postoperative pain and healing following regenerative endodontics using platelet-rich plasma versus conventional endodontic treatment in necrotic mature mandibular molars with chronic periapical periodontitis: a randomized clinical trial. *Int. Endod. J.* **56**, 404–418. https://doi.org/10.1111/iej.13886 (2023).
15. He, L. et al. Treatment of necrotic teeth by apical revascularization: meta-analysis. *Sci. Rep.* **7**, 13941. https://doi.org/10.1038/s41598-017-14412-x (2017).
16. Saoud, T. M. et al. Treatment of mature permanent teeth with necrotic pulps and apical periodontitis using regenerative endodontic procedures: a case series. *J. Endod.* **42**, 57–65. https://doi.org/10.1016/j.joen.2015.09.015 (2016).
17. Youssef, A., Ali, M., ElBolok, A. & Hassan, R. Regenerative endodontic procedures for the treatment of necrotic mature teeth: a preliminary randomized clinical trial. *Int. Endod. J.* **55**, 334–346. https://doi.org/10.1111/iej.13681 (2022).
18. Meschi, N., Palma, P. J. & Cabanillas-Balsera, D. Effectiveness of revitalization in treating apical periodontitis: a systematic review and meta-analysis. *Int. Endod. J.* **56**, 510–532. https://doi.org/10.1111/iej.13778 (2023).
19. Barbosa, W. L. S. et al. Efficacy of pulp revascularization in the treatment of apical periodontitis in mature necrotic teeth: an umbrella review. *Aust. Endod. J.* https://doi.org/10.1111/aej.12928 (2025).

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20. Virdee, S. S., Bashir, N. Z., Grant, M. M., Cooper, P. R. & Tomson, P. L. Therapeutic irrigant procedures for treating apical periodontitis (TIPTAP): a triple-blinded parallel-group randomized controlled phase I/II trial. *Int. Endod. J.* https://doi.org/10.1111/iej.14233 (2025).
21. Abada, H. M., Hashem, A. A. R., Abu-Seida, A. M. & Nagy, M. M. The effect of changing apical foramen diameter on regenerative potential of mature teeth with necrotic pulp and apical periodontitis. *Clin. Oral Investig.* **26**, 1843–1853. https://doi.org/10.1007/s00784-021-04159-1 (2022).
22. Bucchi, C., de-Anta, J. M. & Manzanares-Céspedes, M. C. Micro-computed tomographic analysis of apical foramen enlargement of mature teeth: a cadaveric study. *Int. J. Odontostomat.* **14**, 177–182. https://doi.org/10.4067/S0718-381X2020000200177 (2020).
23. Straub, A. et al. Investigation of three common centrifugation protocols for platelet-rich fibrin (PRF) as a bio-carrier for ampicillin/sulbactam: a prospective trial. *Clin. Oral Investig.* **27**, 5991–5998. https://doi.org/10.1007/s00784-023-05212-x (2023).
24. Akpınar, H. & Ayrancı, F. Is injectable platelet-rich fibrin really effective in reducing expected side effects of removing impacted third molar surgery? *J. Oral Maxillofac. Surg.* **82**, 102–112. https://doi.org/10.1016/j.joms.2023.09.018 (2024).
25. Ørstavik, D., Kerekes, K. & Eriksen, H. M. The periapical index: a scoring system for radiographic assessment of apical periodontitis. *Dent. Traumatol.* **2**, 20–34. https://doi.org/10.1111/j.1600-9657.1986.tb00119.x (1986).
26. Jensen, M. P., Chen, C. & Brugger, A. M. Interpretation of visual analog scale ratings and change scores: a reanalysis of two clinical trials of postoperative pain. *J. Pain* **4**, 407–414. https://doi.org/10.1016/S1526-5900(03)00716-8 (2003).
27. Widbiller, M., Knüttel, H., Meschi, N. & Durán-Sindreu Terol, F. Effectiveness of endodontic tissue engineering in treatment of apical periodontitis: a systematic review. *Int. Endod. J.* **56**, 533–548. https://doi.org/10.1111/iej.13784 (2023).
28. Akyüz, İ. E., Eminsoy Avcı, A. T., Üstün, Y., Kolçakoğlu, K. S. & Kızılcı, E. Evaluation of the effect of different irrigation solutions used in regenerative endodontic treatment of necrotic molar teeth with open apex on postoperative pain-randomized clinical trial. *Clin. Oral Investig.* **29**, 66. https://doi.org/10.1007/s00784-025-06153-3 (2025).
29. Pertek Hatipoğlu, F. et al. Determinants of regenerative endodontic therapy use among pediatric and endodontic specialists in 13 countries. *Sci. Rep.* **15**, 19493. https://doi.org/10.1038/s41598-025-04060-x (2025).

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30. Hatipoğlu, F. P. et al. Attitude and practice of regenerative endodontic procedures among endodontists and paediatric dentists: a multinational survey from 13 countries. *Int. J. Paediatr. Dent.* **33**, 521–534. https://doi.org/10.1111/ipd.13101 (2023).
31. Lasheen, L. R., Gawdat, S. I. & Lutfy, R. A. Success rate of single versus two-visit regenerative treatment protocol in non-vital mature anterior teeth (a preliminary randomised clinical trial). *Aust. Endod. J.* https://doi.org/10.1111/aej.12943 (2025).
32. Abo-Heikal, M. M., El-Shafei, J. M., Shouman, S. A. & Roshdy, N. N. Evaluation of the efficacy of injectable platelet-rich fibrin versus platelet-rich plasma in the regeneration of traumatized necrotic immature maxillary anterior teeth: a randomized clinical trial. *Dent. Traumatol.* **40**, 61–75. https://doi.org/10.1111/edt.12881 (2024).
33. Nageh, M., Ibrahim, L. A., AbuNaeem, F. M. & Salam, E. Management of internal inflammatory root resorption using injectable platelet-rich fibrin revascularization technique: a clinical study with cone-beam computed tomography evaluation. *Clin. Oral Investig.* **26**, 1505–1516. https://doi.org/10.1007/s00784-021-04123-z (2022).

Table Legends

Table 1. Descriptive statistics of the demographic and clinical characteristics of the participants (n=27)

Table 2. Comparison of Changes in PAI and VAS Scores Before and After Treatment at Different Time Points (n=27)

Table 3. Univariate and Multivariate Logistic Regression Analysis of Demographic and Clinical Variables Associated with Clinical Success and Failure (n=27)

Table 4. ROC analysis results of PAI and VAS variables for predicting clinical failure (n=27)

Figure Legends

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Figure 1. Flow diagram illustrating the selection, exclusion, and inclusion of teeth in the study.

Figure 2. Preparation and clinical application of injectable platelet-rich fibrin (i-PRF) during regenerative endodontic treatment. (a) Injectable platelet-rich fibrin obtained following centrifugation using the low-speed protocol, showing the platelet- and leukocyte-rich liquid fraction in the upper portion of the tube. (b) Aspiration of injectable platelet-rich fibrin into a syringe without disturbing the erythrocyte layer. (c) Induction of controlled apical bleeding by extending a sterile #35 K-file beyond the apical foramen to initiate blood clot formation. (d) Placement of injectable platelet-rich fibrin into the root canal system using a syringe under rubber dam isolation.

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Figure 4. Box–violin plots illustrating the distribution of Visual Analog Scale (VAS) pain scores across preoperative and postoperative time points.

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	Frequency	Percentage (%)
Sex		
Male	10	37.0
Female	17	63.0
Tooth number (FDI notation)		
11	6	22.2
12	6	22.2
21	3	11.1
22	2	7.4
23	1	3.7
35	2	7.4
36	3	11.1
37	1	3.7
44	2	7.4
46	1	3.7
Number of roots		
Single-rooted	22	81.5
Multi-rooted	5	18.5
Anatomical location of the tooth		
Maxilla	18	66.7
Mandible	9	33.3
	Mean ± SD	**Median (min: max)**
Age	25.7 ± 9.89	24 (13: 43)
Follow-up duration (months)	22.52 ± 5.52	21 (14: 40)

Values are presented as n (%) or mean ± standard deviation. Percentages were calculated based on the total sample size (n = 27). FDI = Fédération Dentaire Internationale.

Table 2. Comparison of Changes in PAI and VAS Scores Before and After Treatment at Different Time Points (n=27)

Measurement time / Variable	Mean ± SD	Median (min-max)	VAS=0 n (%)	VAS ≥4 n (%)
PAI-Preoperative	4.3 ± 0.78	4 (3–5)		
PAI-Postoperative	1.07 ± 1.57	1 (0–5)		
ΔPAI (Preoperative–Postoperative)	3.22 ± 1.65	3 (–2–5)		
VAS-Preoperative	0.7 ± 1.07	0 (0–3)[ab]	17 (63.0)	0 (0.0)
VAS-6. Hours	0.89 ± 0.93	1 (0–3)[a]	12 (44.4)	0 (0.0)
VAS-12. Hours	0.44 ± 0.58	0 (0–2)[ab]	16 (59.3)	0 (0.0)
VAS-24. Hours	0.37 ± 0.56	0 (0–2)[ab]	18 (66.7)	0 (0.0)
VAS-72. Hours	0.22 ± 0.51	0 (0–2)[ab]	22 (81.5)	0 (0.0)

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VAS-Day 7	0.19 ± 0.48	0 (0–2)[b]	23 (85.2)	0 (0.0)
ΔVAS (VAS Preoperative – VAS Day 7)	0.52 ± 1.05	0 (–2–3)		
Peak postoperative pain†		1 (0–3)		0 (0.0)

*For PAI changes, the Wilcoxon signed-rank test was used; for the VAS time series, the Friedman test was applied. a–b: Measurements sharing the same letter indicate no statistically significant difference. † Peak postoperative pain was defined as the highest VAS score recorded per patient across all postoperative assessments

Table 3. Univariate and Multivariate Logistic Regression Analysis of Demographic and Clinical Variables Associated with Clinical Success and Failure (n=27)

	Treatment outcome		**Univariate**		**Multiple**	
	Success	**Failure**	**OR (95% CI)**	***p***	**OR (95% CI)**	*p*
Sex						
Male	8 (80)	2 (20)		Reference		
Female	16 (94.1)	1 (5.9)	0.25 (0.02: 3.189)	0.286	0.187 (0.012: 2.935)	0.232
Number of roots						
Single-rooted	19 (86.4)	3 (13.6)		Reference		
Multi-rooted	5 (100)	0 (0)	---	---	---	---
Anatomical location of the tooth						
Maxilla	16 (88.9)	2 (11.1)		Reference		
Mandible	8 (88.9)	1 (11.1)	1 (0.078: 12.757)	1.000	0.404 (0.016: 9.976)	0.579
Age	25.96 ± 9.92	23.67 ± 11.55	0.975 (0.857: 1.109)	0.702	0.953 (0.819: 1.11)	0.536
PAI-Preoperative	4.29 ± 0.75	4.33 ± 1.15	1.076 (0.218: 5.317)	0.929	1.177 (0.183: 7.584)	0.864
VAS-Preoperative	0.79 ± 1.1	0 ± 0	---	---	---	---
VAS-6. Hours	0.92 ± 0.93	0.67 ± 1.15	0.726 (0.175: 3.007)	0.659	---	---
VAS-Day 7	0.13 ± 0.34	0.67 ± 1.15	4.591 (0.676: 31.182)	0.119	---	---

Cox & Snell R^2 = 0.063; Nagelkerke R^2 = 0.125; OR (95% CI): Odds Ratio (%95 Confidence Interval); Frequency (percentage); Mean ± Standard deviation

Table 4. ROC analysis results of PAI and VAS variables for predicting clinical failure (n=27)

	AUC (%95 CI)	***p***
PAI-Preoperative	0.542 (0.137: 0.946)	0.817
VAS-Preoperative	0.292 (0.052: 0.531)	0.247
VAS-6. Hours	0.417 (0.041: 0.792)	0.643
VAS-Day 7	0.625 (0.230–1.000)	0.487
ΔVAS (Preoperative – Day 7)	0.194 (0: 0.439)	0.090

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AUC (95% CI): Area under the curve (95% Confidence Interval)

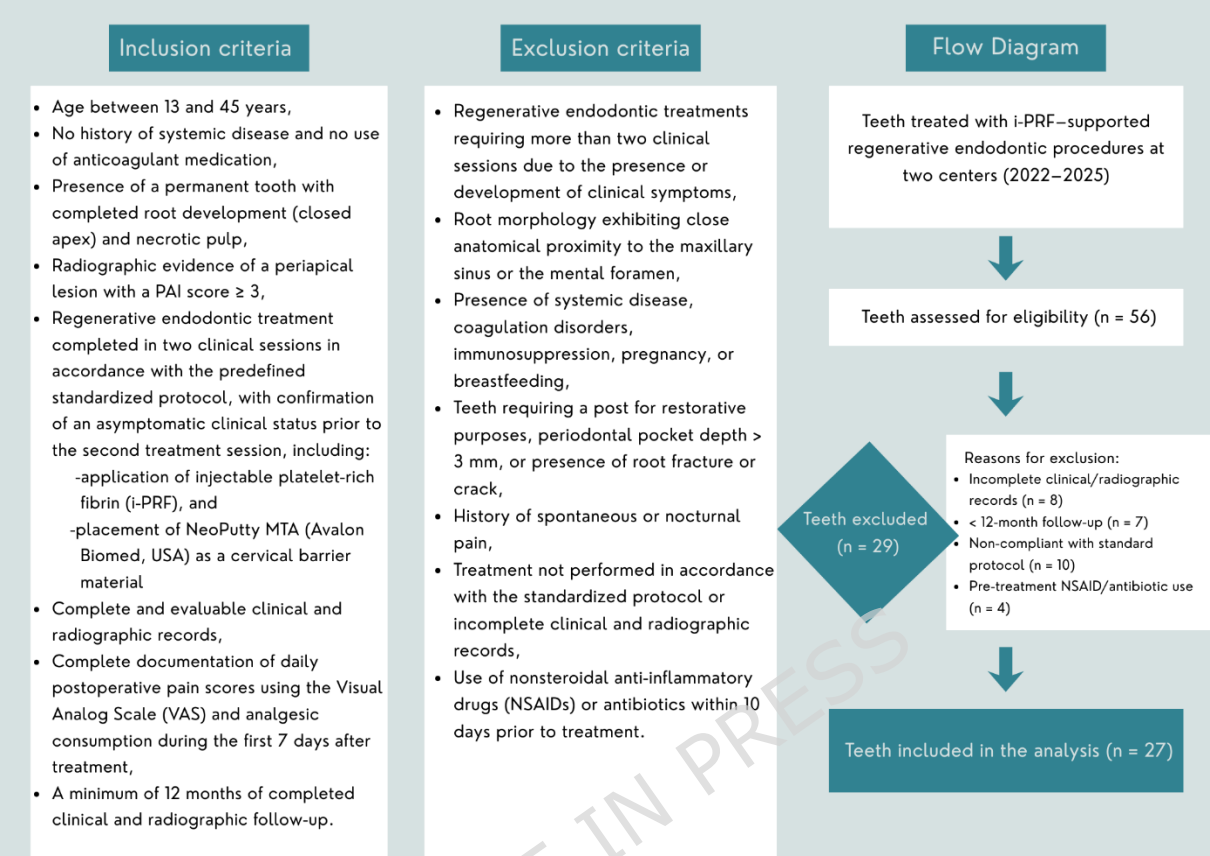


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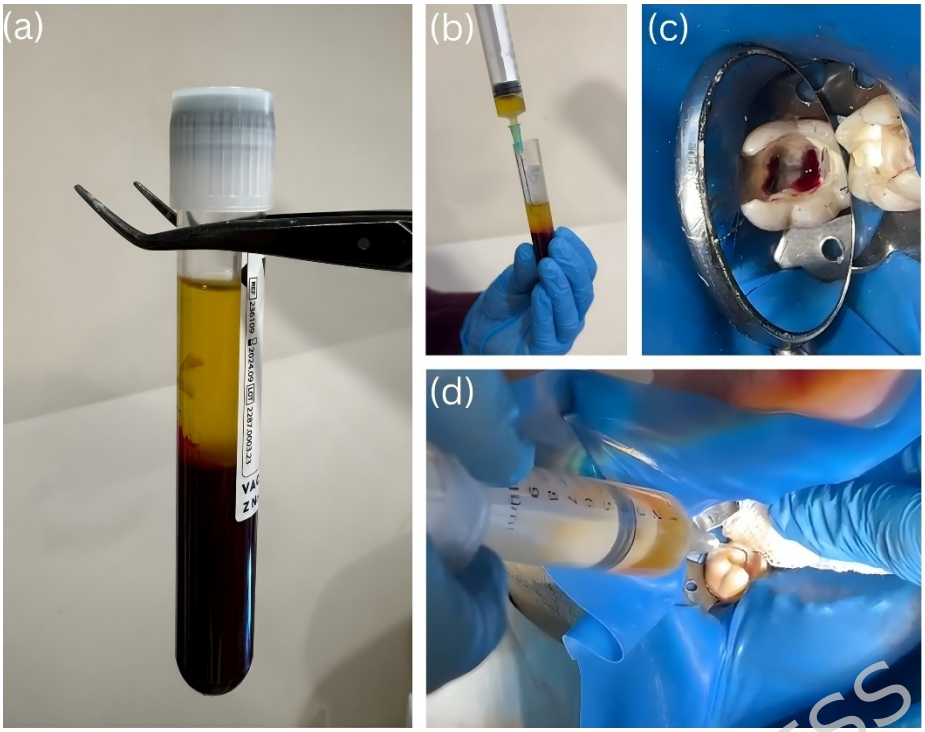


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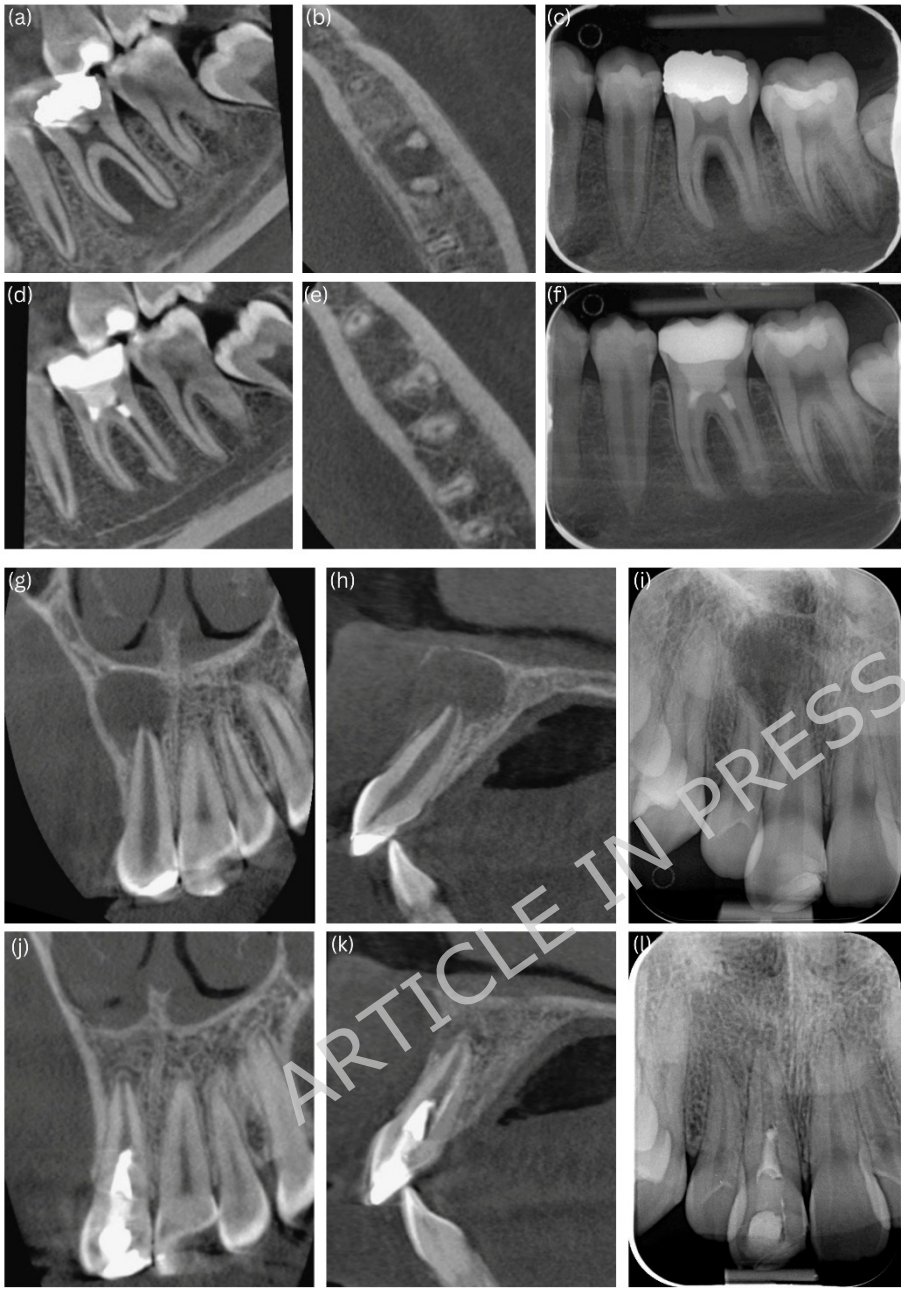


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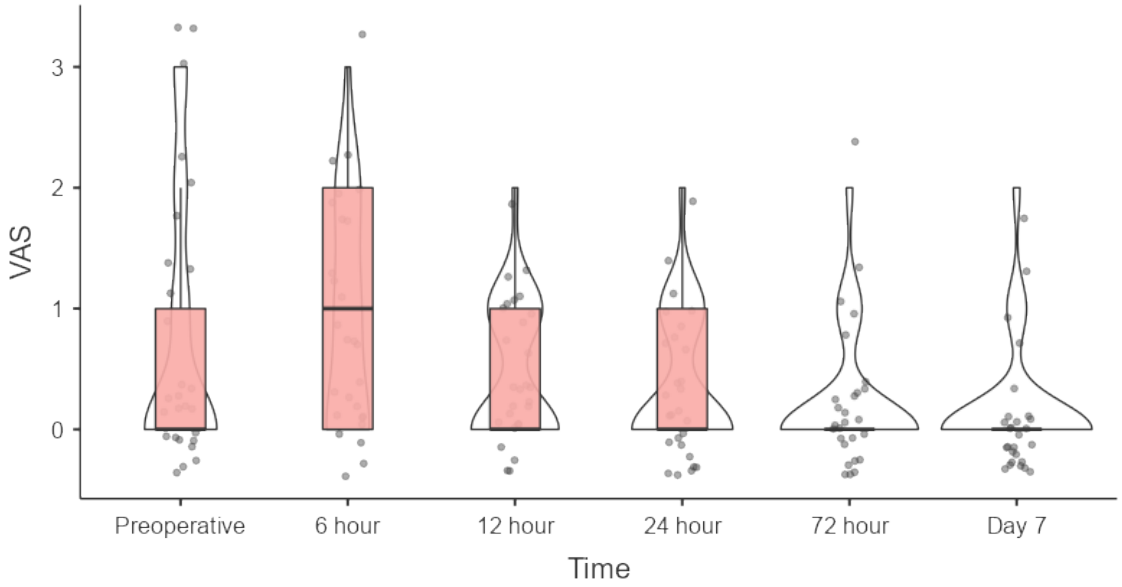


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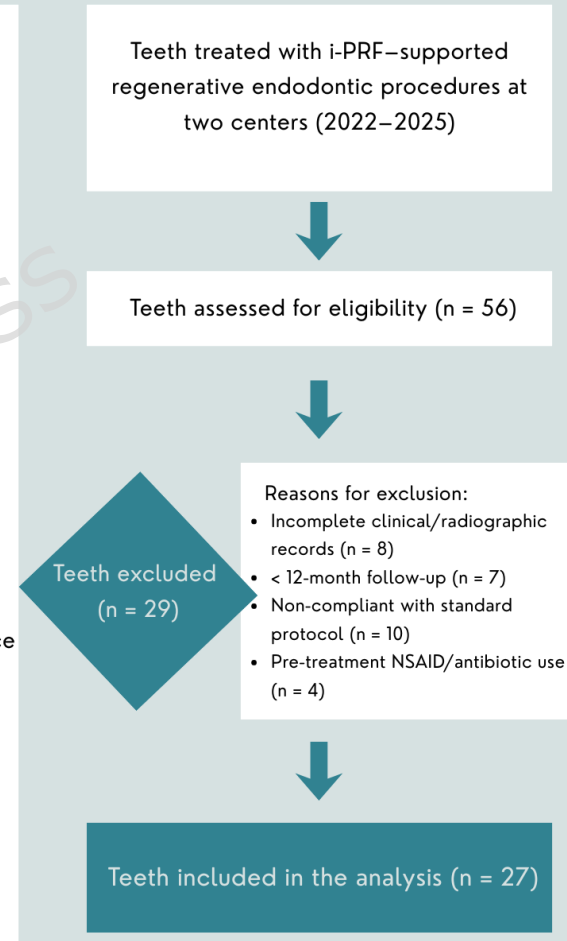
Inclusion criteria

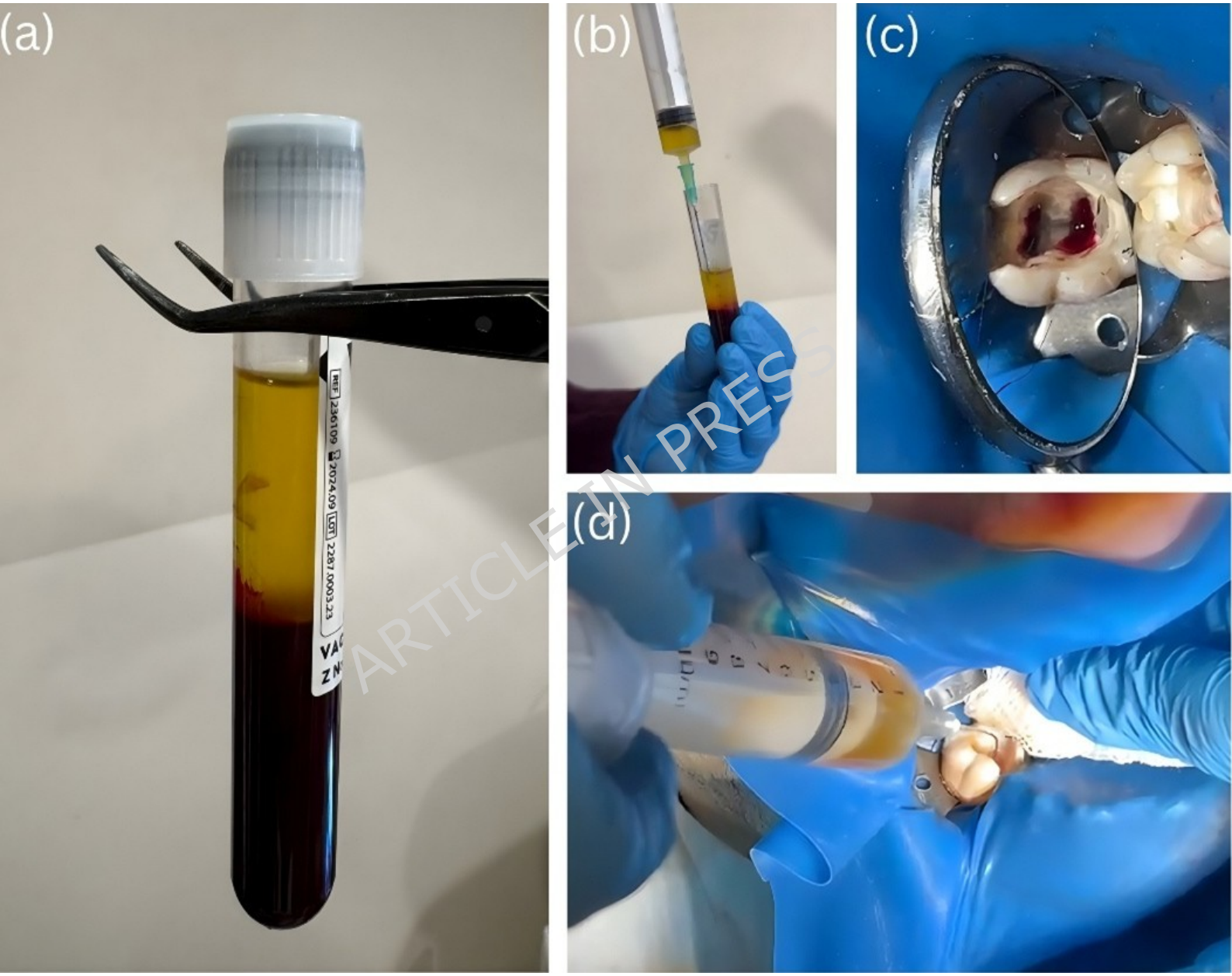
- Age between 13 and 45 years,
- No history of systemic disease and no use of anticoagulant medication,
- Presence of a permanent tooth with completed root development (closed apex) and necrotic pulp,
- Radiographic evidence of a periapical lesion with a PAI score ≥ 3,
- Regenerative endodontic treatment completed in two clinical sessions in accordance with the predefined standardized protocol, with confirmation of an asymptomatic clinical status prior to the second treatment session, including:
  - -application of injectable platelet-rich fibrin (i-PRF), and
  - -placement of NeoPutty MTA (Avalon Biomed, USA) as a cervical barrier material
- Complete and evaluable clinical and radiographic records,
- Complete documentation of daily postoperative pain scores using the Visual Analog Scale (VAS) and analgesic consumption during the first 7 days after treatment,
- A minimum of 12 months of completed clinical and radiographic follow-up.

Exclusion criteria

- Regenerative endodontic treatments requiring more than two clinical sessions due to the presence or development of clinical symptoms,
- Root morphology exhibiting close anatomical proximity to the maxillary sinus or the mental foramen,
- Presence of systemic disease, coagulation disorders, immunosuppression, pregnancy, or breastfeeding,
- Teeth requiring a post for restorative purposes, periodontal pocket depth > 3 mm, or presence of root fracture or crack,
- History of spontaneous or nocturnal pain,
- Treatment not performed in accordance with the standardized protocol or incomplete clinical and radiographic records,
- Use of nonsteroidal anti-inflammatory drugs (NSAIDs) or antibiotics within 10 days prior to treatment.

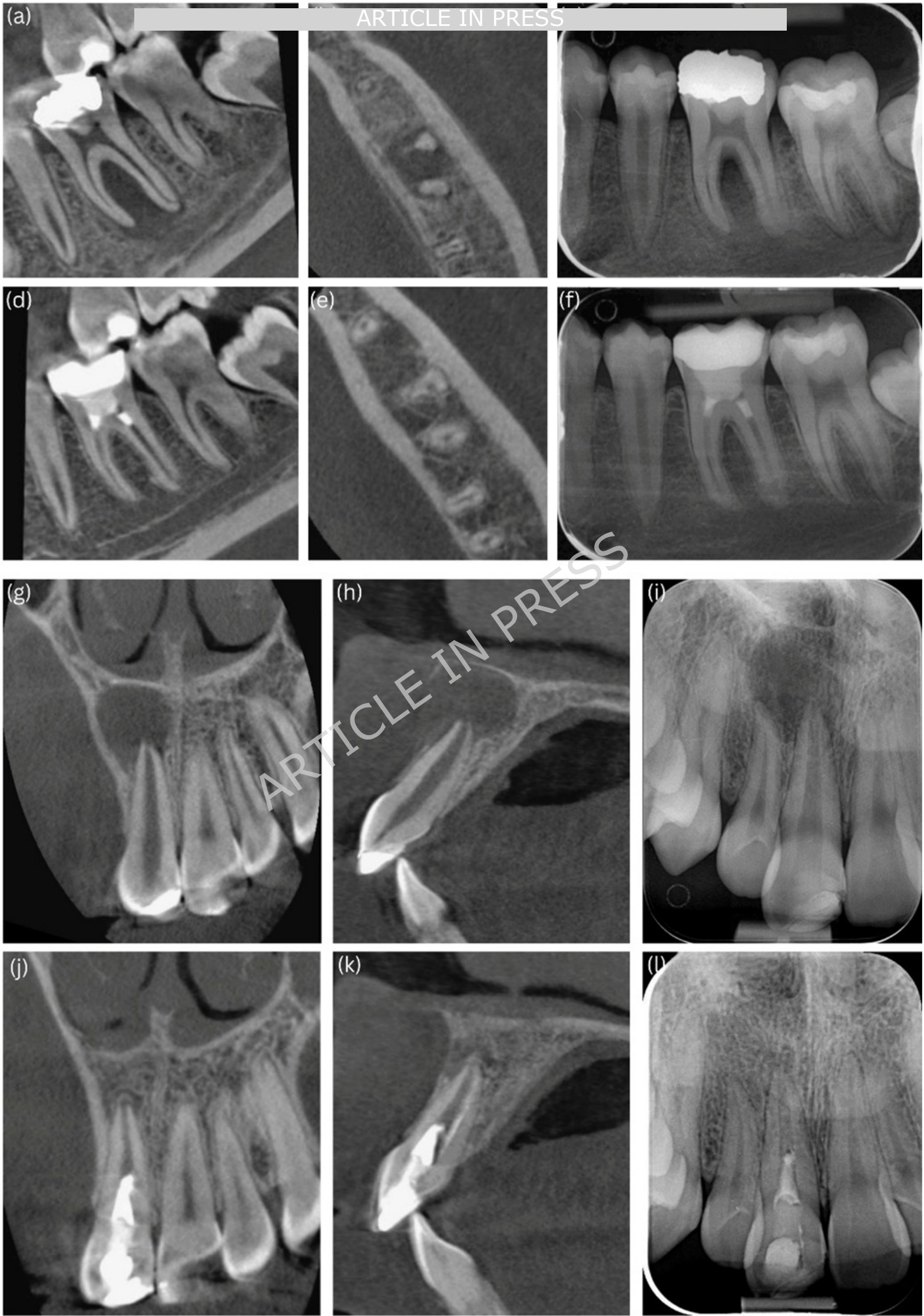
Flow Diagram





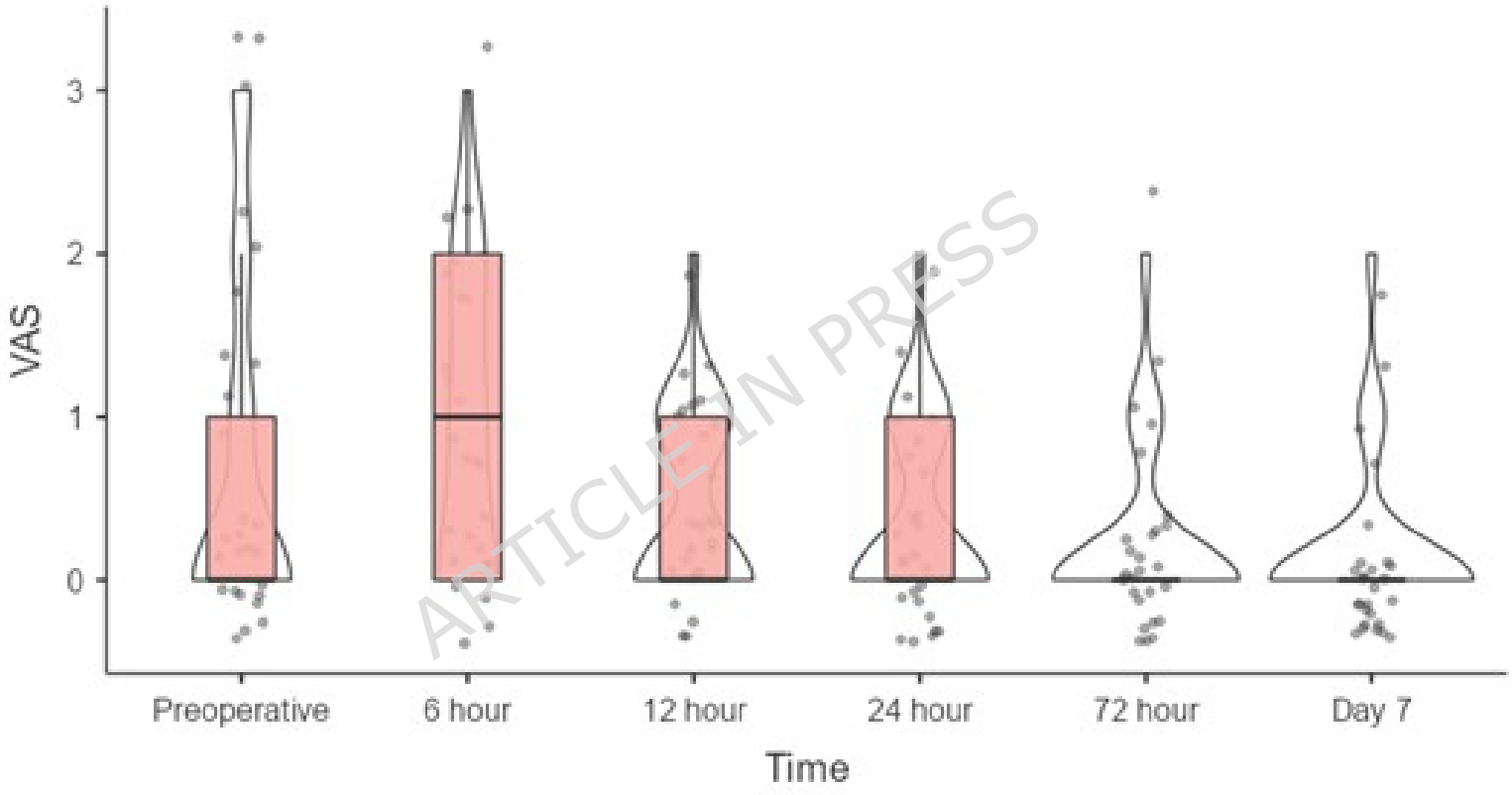
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*For PAI changes, the Wilcoxon signed-rank test was used; for the VAS time series, the Friedman test was applied. a–b: Measurements sharing the same letter indicate no statistically significant difference. † Peak postoperative pain was defined as the highest VAS score recorded per patient across all postoperative assessments.

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Table 3. Univariate and Multivariate Logistic Regression Analysis of Demographic and Clinical Variables Associated With Clinical Success and Failure (n=27)

		Treatment outcome		Univariate		Multiple	
		Success	**Failure**	**OR (95% CI)**	**p**	**OR (95% CI)**	p
Sex							
	Male	8 (80)	2 (20)		Reference		
	Female	16 (94.1)	1 (5.9)	0.25 (0.02: 3.189)	0.286	0.187 (0.012: 2.935)	0.232
Number of roots							
	Single-rooted	19 (86.4)	3 (13.6)		Reference		
	Multi-rooted	5 (100)	0 (0)	---	---	---	---
Anatomical location of the tooth							
	Maxilla	16 (88.9)	2 (11.1)		Reference		
	Mandible	8 (88.9)	1 (11.1)	1 (0.078: 12.757)	1.000	0.404 (0.016: 9.976)	0.579
Age		25.96 ± 9.92	23.67 ± 11.55	0.975 (0.857: 1.109)	0.702	0.953 (0.819: 1.11)	0.536
PAI-Preoperative		4.29 ± 0.75	4.33 ± 1.15	1.076 (0.218: 5.317)	0.929	1.177 (0.183: 7.584)	0.864
VAS-Preoperative		0.79 ± 1.1	0 ± 0	---	---	---	---
VAS-6. Hours		0.92 ± 0.93	0.67 ± 1.15	0.726 (0.175: 3.007)	0.659	---	---
VAS-Day 7		0.13 ± 0.34	0.67 ± 1.15	4.591 (0.676: 31.182)	0.119	---	---

Cox & Snell R^2 = 0.063; Nagelkerke R^2 = 0.125; OR (95% CI): Odds Ratio (%95 Confidence Interval); Frequency (percentage); Mean ± Standard deviation

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Table 4. ROC analysis results of PAI and VAS variables for predicting clinical failure (n=27)

	AUC (%95 CI)	p
PAI-Preoperative	0.542 (0.137: 0.946)	0.817
VAS-Preoperative	0.292 (0.052: 0.531)	0.247
VAS-6. Hours	0.417 (0.041: 0.792)	0.643
VAS-Day 7	0.625 (0.230–1.000)	0.487
ΔVAS (Preoperative – Day 7)	0.194 (0: 0.439)	0.090

AUC (95% CI): Area under the curve (95% Confidence Interval)

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