PRELIMINARY REPORT

UDC: 576.3:617-089.843]::616.314-08
DOI: https://doi.org/10.2298/VSP260114025K

Clinical and radiographic outcomes of autologous pulp transplantation enhanced with concentrated growth factor in mature necrotic teeth: a clinical study

Klinički i radiografski ishodi autologne transplantacije pulpe unapređene koncentrovanim faktorom rasta kod stalnih nekrotičnih zuba: klinička studija

Aliye Kamalak, Esra Balkanlioğlu, Elife Ülkü Tatar

Kahramanmaraş Sütçü İmam University, Faculty of Dentistry, Department of Endodontics, Kahramanmaraş, Türkiye

Abstract

Background/Aim. Regenerative endodontic therapy in mature teeth remains challenging due to limited stem cell recruitment and apical vascularization. The aim of the prospective pilot clinical study was to evaluate the feasibility and preliminary clinical, radiographic, and functional outcomes of autologous pulp transplantation enhanced with concentrated growth factor (CGF) in mature necrotic permanent teeth. **Methods.** The study included six systemically healthy patients (five females and one male, aged 18–27 years) presenting with single-rooted mature permanent teeth, diagnosed with pulp necrosis and associated periapical lesions. Autologous pulp tissue was harvested from extracted third molars and transplanted into disinfected recipient root canals in combination with CGF prepared from venous blood. Clinical and radiographic evaluations were performed at 3, 6, and 12 months. Periapical healing was assessed using cone-beam computed tomography (CBCT), while pulp sensibility was evaluated using electric pulp testing (EPT). Treatment outcome measures included postoperative symptoms, tooth function, radiographic healing, and recovery of pulp sensibility. **Results.** All treated teeth (6/6) remained asymptomatic and functional throughout the 12-month follow-up period, with no postoperative complications, such as pain, swelling, sinus tract formation, or abnormal mobility. Progressive recovery of pulp sensibility was observed during follow-up, and positive EPT responses (reflecting neural responsiveness) were detected in two cases at 4 months, one case at 6 months, and in all cases by the 12-month evaluation. CBCT analysis at 12 months demonstrated complete resolution of periapical radiolucency and restoration of apical bone architecture in all treated teeth (6/6). **Conclusion.** Within the limitations of this pilot clinical study, CGF-enhanced autologous pulp transplantation was associated with favorable short-term clinical and radiographic outcomes, as well as recovery of pulp sensibility in mature necrotic teeth. These preliminary findings suggest the potential of this biologically based regenerative approach. However, comparative effectiveness remains to be established.

Keywords:
biological factors; dental pulp; regenerative endodontics; stem cells; transplantation, autologous.

Apstrakt

Uvod/Cilj. Regenerativna endodontska terapija kod stalnih zuba predstavlja izazov zbog ograničene migracije matičnih ćelija i nedovoljne apikalne vaskularizacije. Cilj ove prospektivne pilot kliničke studije bio je da se procene izvodljivost i preliminarni klinički, radiografski i funkcionalni ishodi autologne transplantacije pulpe unapređene koncentrovanim faktorom rasta (*concentrated growth factor* – CGF) kod stalnih nekrotičnih zuba. **Metode.** Studijom je obuhvaćeno šest sistemski zdravih pacijenata (pet ženskog i jedan muškog pola, starosti 18–27 godina) sa jednokorenim stalnim zubima, kod kojih je dijagnostikovana nekroza pulpe sa pratećim periapikalnim lezijama. Autologno pulpno tkivo uzeto je iz ekstrahovanih trećih molara i transplantirano u dezinfikovane kanale korena recipijentnih zuba u kombinaciji sa CGF dobijenim iz venske krvi. Kliničke i radiografske procene sprovedene su nakon 3, 6 i 12 meseci. Periapikalno zarastanje procenjivano je pomoću kompjuterizovane tomografije konusnog zraka (*cone-beam computed tomography* – CBCT), dok je osetljivost pulpe procenjivana električnim testiranjem pulpe (*electric pulp testing* – EPT). Mere ishoda lečenja uključivale su postoperativne simptome, funkciju zuba, radiografsko zarastanje i oporavak osetljivosti pulpe. **Rezultati.** Svi lečeni zubi (6/6) ostali su asimptomatski i funkcionalni tokom 12 meseci praćenja, bez postoperativnih komplikacija kao što su bol, otok,

Correspondence to: Aliye Kamalak, Kahramanmaraş Sutcu Imam University, Faculty of Dentistry, Department of Endodontics, Avsar Campus West Ring Road Boulevard No: 251/A Onikisubat/Kahramanmaraş, Türkiye. E-mail: aliyekamalak@gmail.com

formiranje sinusnog trakta ili abnormalna pokretljivost. Tokom praćenja zabeležen je progresivan oporavak pulpne osetljivosti, a pozitivni EPT odgovori (koji odražavaju osetljivost nerva) otkriveni su u dva slučaja posle 4 meseca, u jednom slučaju posle 6 meseci i u svim slučajevima do kraja 12. meseca. Primenom CBCT nakon 12 meseci pokazana je potpuna rezolucija periapikalnog rasvetljenja i obnova apikalne koštane strukture u svim lečenim zubima (6/6). **Zaključak.** Uzimajući u obzir ograničenja ove pilot kliničke studije, autologna transplantacija pulpe unapređena primenom CGF bila je povezana sa povoljnijim kratkoročnim kliničkim i radiografskim ishodima, kao i sa oporavakom pulpne osetljivosti kod stalnih nekrotičnih zuba. Ovi preliminarni nalazi ukazuju na potencijal tog biološki zasnovanog regenerativnog pristupa. Međutim, uporedna efikasnost tek treba da bude utvrđena.

Ključne reči:
biološki faktori; zub, pulpa; endodoncija, regenerativna; matične ćelije; transplantacija, autologna.

Introduction

Loss of pulp vitality and periapical infection remain major causes of tooth dysfunction and eventual extraction. Despite substantial advances in conventional endodontic treatment, non-vital teeth continue to be at risk for long-term structural compromise and reinfection. Regenerative endodontic procedures (REPs) were developed to biologically restore the structure and function of the pulp–dentin complex rather than merely obturate the root canal space [1]. This paradigm shift in endodontics aims to reestablish pulp vitality, enhance innate defense mechanisms, and preserve long-term tooth integrity.

Initially introduced for immature permanent teeth, regenerative approaches have recently been extended to mature teeth. Notably, mature teeth are more likely to respond positively to electric pulp testing (EPT) than immature ones (45% vs. 25%), suggesting that mature necrotic teeth may regain pulp sensibility under appropriate biological conditions [2]. Clinical and radiographic evidence indicates that REPs in mature permanent teeth can lead to healing of apical periodontitis and symptom resolution [3, 4]. However, achieving reproducible outcomes in mature necrotic teeth remains challenging due to limited stem cell (SC) migration, reduced apical vascularization, and constrained apical pathways [5].

To address these biological constraints, research has focused on developing biologically driven strategies that promote angiogenesis, neurogenesis, and reinnervation within the canal system. Cell-based regenerative endodontic therapies have been proposed as a promising approach to achieve biologically relevant regeneration [6]. However, the clinical translation of such methods into daily clinical practice is restricted by the need for good manufacturing practice-compliant facilities, SC banking, regulatory constraints, and high costs [7]. Consequently, there is a growing demand for simplified, autologous, and chairside-feasible regenerative approaches.

Pulp tissue transplantation, as a biologically sound alternative, utilizes autologous dental pulp SCs (DPSCs) within their native extracellular matrix, thereby eliminating the need for *ex vivo* cell expansion [8]. Favorable clinical outcomes have been reported when pulp tissue harvested from extracted third molars (TMs) or primary teeth was transplanted into necrotic teeth, demonstrating periapical healing and restored sensibility [9, 10]. These findings highlight pulp transplantation as a clinically applicable cell-based regenerative strategy.

Concentrated growth factor (CGF), the most recent generation of autologous platelet concentrates, represents an additional innovation in regenerative dentistry. CGF provides a dense fibrin scaffold with a sustained release of bioactive molecules such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) [11–13]. These growth factors can enhance cellular proliferation, angiogenesis, and differentiation of human dental pulp cells, thereby accelerating tissue healing and regeneration [12]. Incorporating CGF into pulp transplantation may thus enhance the biological microenvironment for successful revitalization of mature necrotic teeth [14]. Although CGF has demonstrated promising biological and preclinical effects, clinical evidence supporting CGF-assisted pulp transplantation in mature necrotic teeth remains limited, underscoring the need for investigations like the present study.

Therefore, this pilot clinical study aimed to evaluate the feasibility and preliminary clinical and radiographic healing and functional outcomes of autologous pulp transplantation enhanced with CGF in mature necrotic teeth.

Methods

This study was designed as a pilot feasibility clinical investigation rather than a hypothesis-testing trial. The study was approved by the Kahramanmaraş Sutcu Imam University Clinical Research Ethics Committee (No. 2025-KAEK-53) and conducted in accordance with the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (Identifier: NCT07314866). Registration was completed after recruitment had commenced in order to ensure transparency and public accessibility of the study protocol. The study design, inclusion criteria, and intervention protocol were predefined prior to patient enrollment, and no modifications were made after registration. Donor TMs were extracted only when they were already indicated for clinical removal as part of routine dental care; no tooth extraction was performed solely for the purposes of this study. Before participating, all individuals received a comprehensive briefing on the study's objectives, the available treatment options, and the

potential risks, following which written informed consent was obtained. To ensure consistency and standardization of clinical procedures, all were performed by a single endodontist with over 10 years of clinical experience at the Department of Endodontics, Faculty of Dentistry, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Türkiye.

Patient selection and preoperative evaluation

Six systemically healthy patients (five females and one male, aged 18–27 years) were prospectively enrolled and treated according to a predefined clinical protocol at the Kahramanmaraş Sutcu Imam University Endodontics Clinic in 2025. Eligible participants were systemically healthy individuals presenting with single-rooted mature permanent teeth exhibiting clinical and radiographic evidence of pulp necrosis associated with periapical radiolucency.

Teeth were required to have a probing depth of less than 3 mm, no abnormal mobility, and no history of previous endodontic or apical surgery. The study included only those patients for whom autologous donor pulp tissue could be obtained from their own impacted or nonfunctional TMs, already clinically indicated for extraction. No donor tooth was extracted solely for research purposes or for pulp harvesting. Patients were excluded if they had systemic diseases or conditions that could impair healing (e.g., diabetes mellitus, autoimmune disorders, or ongoing immunosuppressive therapy), a history of radiation therapy in the head and neck region, or pregnancy.

Teeth with root fractures, internal or external resorption, severe calcification, or nonrestorable crown destruction were also excluded. Patients with poor oral hygiene, deep periodontal pockets (> 3 mm), or those unable to comply with follow-up visits were not considered eligible.

A formal sample size calculation was not performed, as the study was not intended to test statistical superiority or comparative efficacy. The final sample size (n = 6) reflects consecutive eligible patients meeting strict inclusion criteria during the recruitment period. Recruitment was limited by the requirement for suitable donor TMs and patient consent for autologous pulp harvesting, which inherently restricted the eligible population.

All cases were examined clinically and radiographically using periapical radiographs and cone-beam computed tomography (CBCT) to assess baseline periapical radiolucency, root morphology, and apical patency.

Clinical procedures

All treatments were performed by a single experienced endodontist following a standardized two-visit protocol (Figure 1).

First appointment (canal disinfection)

After anesthesia with articaine hydrochloride and epinephrine (Maxicaine®, Vem Pharma, Türkiye), the teeth were isolated with a rubber dam. Straight-line access was

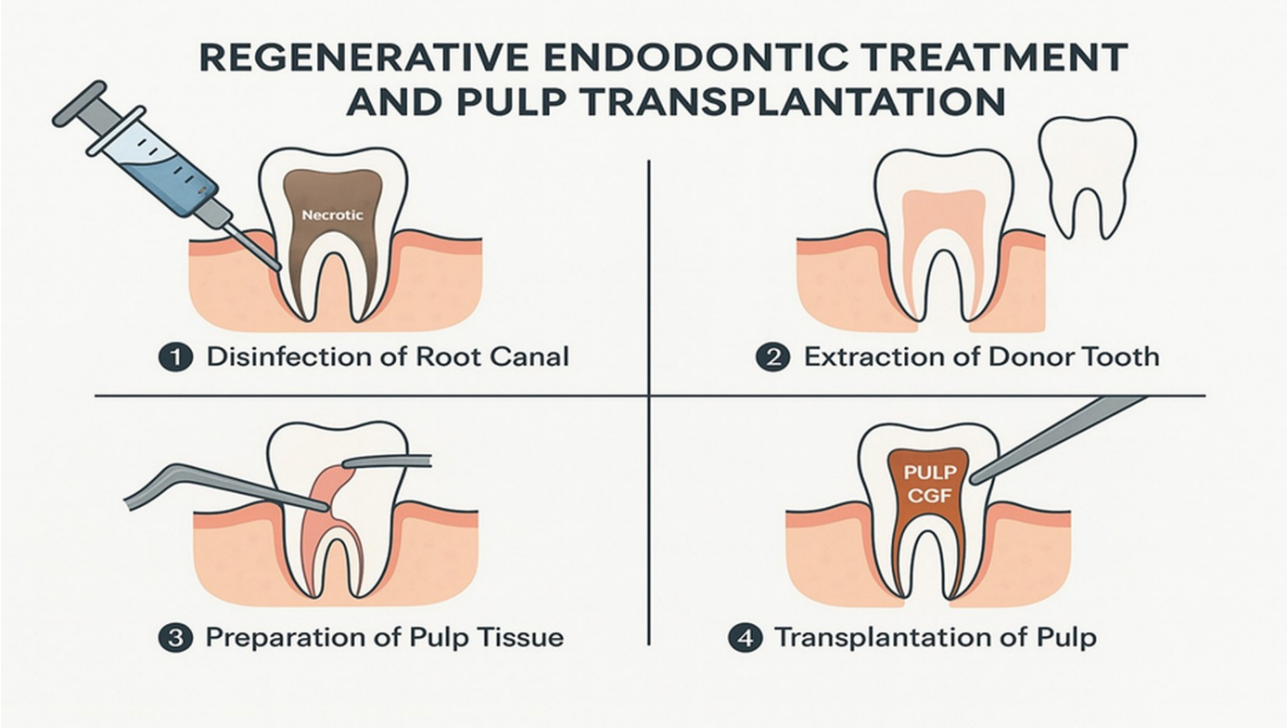

Fig. 1 – Regenerative endodontic treatment and pulp transplantation schematic (AI-assisted illustration). CGF – concentrated growth factor.

created, and the working length was determined using an electronic apex locator (Raypex 6®, VDW, Germany) and confirmed radiographically. Root canals were prepared with stainless-steel K-files (#45–#80) using a step-back technique. However, apical instrumentation was limited to #25–#30 to maintain an apical foramen diameter of approximately 0.25–0.30 mm. During instrumentation, 2 mL of 2.5% sodium hypochlorite (NaOCl) (Microvem®, Türkiye) was used between each file and activated for 5 min using a sonic device (EndoActivator®, Dentsply Sirona, Germany), totaling 20 mL *per* canal. Sonic activation was used to enhance irrigant penetration while minimizing cytotoxic effects. Canals were dried with sterile paper points and medicated with a triple antibiotic paste consisting of cefuroxime, metronidazole, and ciprofloxacin (1 : 1 : 1). Minocycline was intentionally excluded due to its well-documented association with tooth discoloration in REPs [15, 16]. Therefore, cefuroxime was used as an alternative antibiotic to maintain broad-spectrum antimicrobial coverage while reducing the risk of esthetic complications. Temporary restoration was performed using glass-ionomer cement (GC Fuji II LC®, Tokyo, Japan).

Second appointment (pulp transplantation and concentrated growth factor preparation)

After weeks, if no signs of infection were observed, pulp transplantation was initiated. The donor TM was extracted under local anesthesia and stored in sterile saline. The recipient tooth was re-anesthetized with 3% mepivacaine and isolated with a rubber dam.

The temporary material was removed, and the canal was irrigated with 5 mL of sterile saline, 5 mL of 2.5% NaOCl, and 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (MD-Cleanser®, Meta-Biomed, Korea). A #25 K-file was advanced 2 mm beyond the apical foramen to induce controlled bleeding, not to create a scaffold, but to provide the essential oxygenation and nutrient supply required for the survival of the transplanted pulp SCs.

The donor tooth was sectioned under saline cooling, and the pulp tissue was gently removed and trimmed to fit the dimensions of the recipient canal. Immediately after extraction, the donor tooth was stored in sterile saline, and pulp harvesting was performed promptly under continuous saline cooling. The harvested pulp tissue was kept moist and transplanted into the recipient canal without delay. The extraoral period from pulp harvesting to transplantation was limited to approximately 15 min in all cases in order to minimize ischemic injury and preserve tissue viability.

Venous blood was collected into red-top tubes and centrifuged in a MediFuge® (Silfradent, Italy) using the following program: 30 s acceleration; 2 min at 2,700 revolutions *per* minute (rpm) (600 × g); 4 min at 2,400 rpm (400 × g); 4 min at 2,700 rpm (600 × g); 3 min at 3,000 rpm (600 × g); and 36 s deceleration. The middle fibrin layer (CGF) was collected as a soft, elastic gel and placed over the transplanted pulp tissue inside the canal.

A small piece of Spongostan® was placed above the pulp–CGF complex as a matrix for mineral trioxide aggregate (MTA) (ProRoot MTA®, Dentsply Sirona, Germany), which was applied as a 3–4 mm coronal plug positioned 2–3 mm below the cemento-enamel junction. The coronal seal was completed using glass ionomer (Fuji II LC®, GC Corp., Japan) and composite resin (Universal Restorative 200®, 3M ESPE, Germany) (Figure 2).

Outcome measures and definition of success

The primary outcome of this pilot clinical study was radiographic periapical healing at 12 months, assessed by CBCT. Periapical healing was defined as the resolution of periapical radiolucency and restoration of normal apical bone architecture.

Secondary outcomes included the following: recovery of pulp sensibility, assessed by EPT; absence of clinical symptoms, including pain, swelling, sinus tract formation, or abnormal mobility; tooth survival and maintenance of functional integrity throughout the follow-up period.

Treatment success was defined as the presence of complete periapical healing on CBCT combined with the absence of clinical symptoms at 12 months. Recovery of pulp sensibility was considered a secondary functional outcome rather than a mandatory criterion for overall success.

Follow-up and data analysis

Patients were monitored at 3-month intervals for 12 months. Clinical evaluation included assessment of pain, swelling, tenderness, gingival condition, and tooth function. Radiographic healing was assessed by CBCT at 12 months. CBCT scans were obtained using a standardized protocol (90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm). Images were reconstructed in axial, sagittal, and coronal planes for evaluation of periapical healing. CBCT images were evaluated by the treating endodontist with over ten years of clinical experience. Healing was assessed by comparing baseline and 12-month CBCT images and was defined by resolution or marked reduction of periapical radiolucency, re-establishment of trabecular bone pattern within the lesion area, and restoration of apical cortical continuity, when applicable. Images were reviewed in multiplanar reconstructions (axial, sagittal, and coronal planes). Blinded assessment and intra-examiner reliability analysis were not performed, which represents a methodological limitation of this pilot study. Pulp sensibility was tested with EPT at 4, 6, and 12 months.

Given the limited sample size and the exploratory nature of this study, no inferential statistical analyses were performed. Data were analyzed descriptively using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as means ± standard deviations, and categorical variables as percentages.

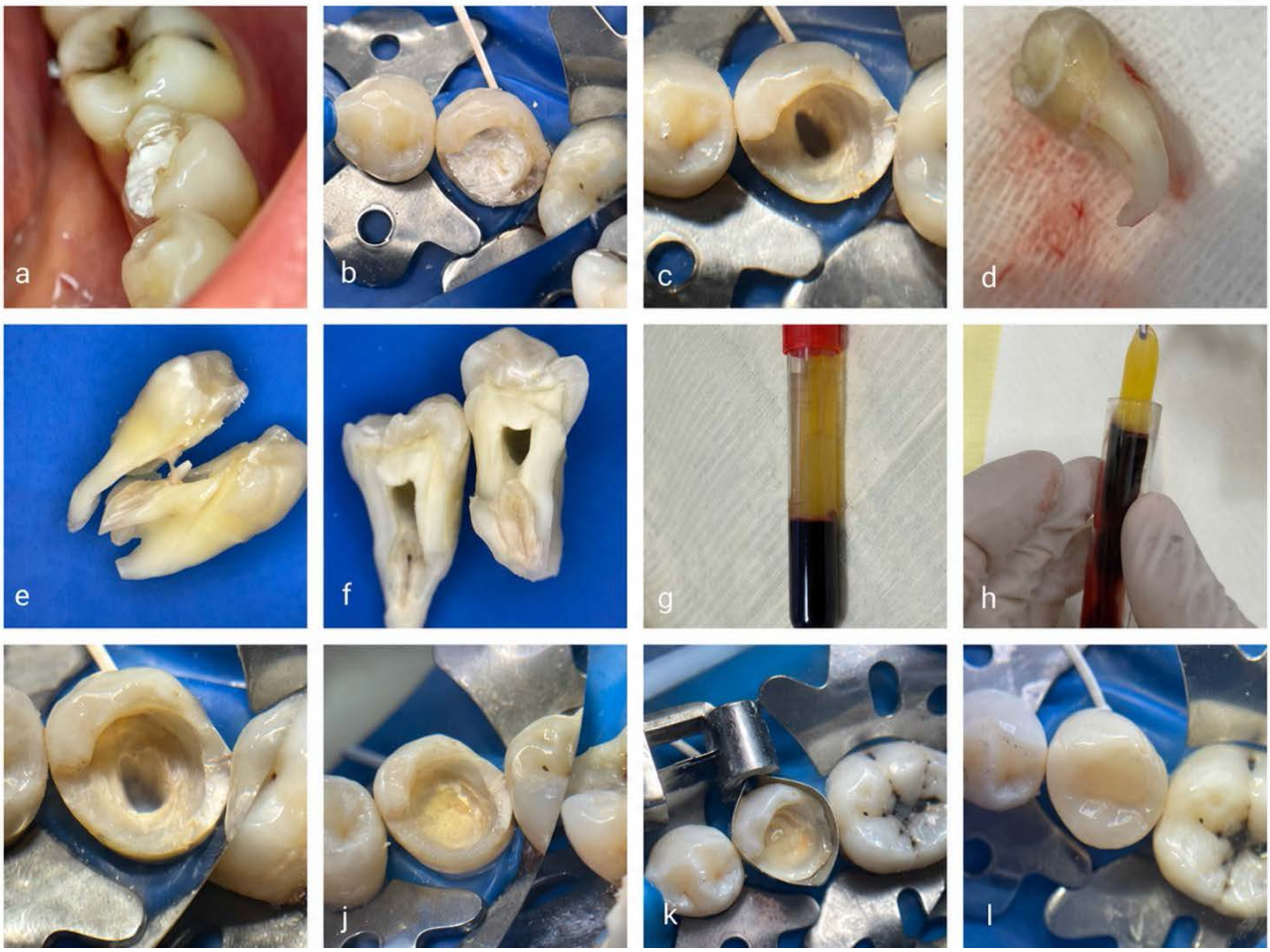

Fig. 2 – Clinical stages of autologous pulp transplantation and regenerative endodontic treatment: a) preoperative intraoral view of the lower left second premolar; b) rubber dam isolation and preparation of the recipient tooth at the second appointment; c) canal preparation for transplantation following removal of the intracanal medicament and irrigation with saline and sodium hypochlorite; d) extraction of the upper left third molar used as the donor tooth; e) sectioning of the donor tooth under saline cooling; f) harvested pulp tissue obtained from the donor tooth; g) concentrated growth factor (CGF) prepared from the patient's venous blood; h) pulp tissue combined with CGF prior to placement into the root canal; i) placement of mineral trioxide aggregate (MTA) as a coronal barrier, 3 mm below the cemento-enamel junction; j) application of a glass ionomer base over the MTA; k) matrix band placement for coronal restoration; and l) final composite resin restoration of the recipient tooth.

Results

Six patients (five females and one male, with a mean age of 21.0 ± 3.7 years) who underwent CGF-enhanced DPSCs were evaluated. All patients were systemically healthy and presented with necrotic permanent teeth associated with periapical radiolucency. Donor pulp tissues were harvested from extracted TMs in all cases. Demographic characteristics, treated teeth, pulp sensibility responses, and radiographic outcomes are summarized in Table 1. In one patient, two adjacent teeth (#22 and #23) were treated during the same clinical session. These teeth are, therefore, presented as separate cases in Table 1. An overview of the clinical procedure is shown in Figure 1.

At baseline, the treated teeth presented with clinical symptoms such as pain, tenderness to percussion, swelling, or discoloration, along with radiographic evidence of periapical bone loss. Following treatment, all patients remained asymptomatic throughout the 12-month follow-up period. No postoperative complications, swelling, sinus tract formation, or abnormal mobility were observed. All teeth remained functional, and coronal restorations were intact at all recall visits.

EPT demonstrated a progressive recovery of pulpal sensibility. Positive EPT responses were observed at 4 months in two cases, at 6 months in one case, and at 12 months in three cases. All EPT-positive teeth were clinically asymptomatic, suggesting recovery of pulp sensibility.

CBCT evaluation at 12 months revealed complete periapical bone healing in all cases (6/6). According to the predefined primary outcome (radiographic healing at 12 months) and success criteria (absence of clinical symptoms and periapical healing), treatment success was achieved in all cases (6/6). Radiographic assessment demonstrated

Table 1

Clinical summary of concentrated growth factor -enhanced pulp transplantation cases

Case No.	Age, years/ Sex	Recipient tooth, #	Donor tooth	Initial symptoms	Preoperative diagnosis	EPT response at 4-6-12 months	CBCT result at 12 months	Notes
1	20/female	35	upper left TM	percussion pain	necrosis, periapical lesion	positive at 12	complete healing	no symptoms during follow-up
2	23/male	22	upper left TM	pain	necrosis, periapical lesion	positive at 6	complete healing	no adverse events
3	27/female	31	upper left TM	swelling, pain, discoloration	calcific metamorphosis, necrosis	positive at 12	complete healing	delayed response, possible trauma effect
4	20/female	21	upper right TM	percussion pain, swelling	necrosis, periapical lesion	positive at 12	complete healing	routine check-ups, no complications
5	18/female	22/23	upper left TM	fractures, mild percussion pain	necrosis, periapical lesions on #22 and #23	positive at 4–6–12	slower but complete healing	gingivectomy and composite lamination may have delayed healing
6	18/female	22/23	upper left TM	fractures, mild percussion pain	necrosis, periapical lesions on #22 and #23	positive at 4–6–12	slower but complete healing	gingivectomy and composite lamination may have delayed healing

EPT– electric pulp testing; CBCT – cone-beam computed tomography; TM – third molar.
Note: **# symbol used to identify a specific tooth number in a patient's mouth.**

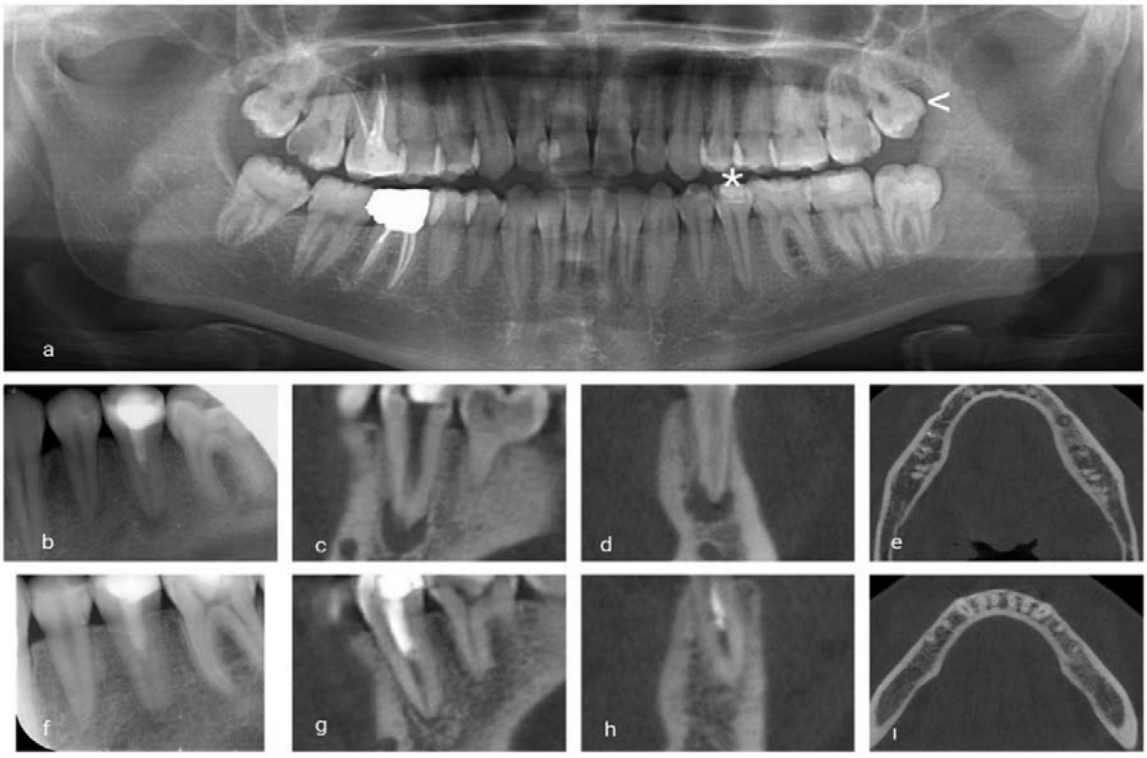

Fig. 3 – Clinical stages and radiographic outcomes of concentrated growth factor-enhanced autologous pulp transplantation (Case 1): a) preoperative panoramic radiograph showing the recipient tooth and donor third molar; b–e) preoperative cone-beam computed tomography (CBCT) images; f–i) CBCT images obtained at the 12-month follow-up demonstrating resolution of periapical radiolucency and bone regeneration. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

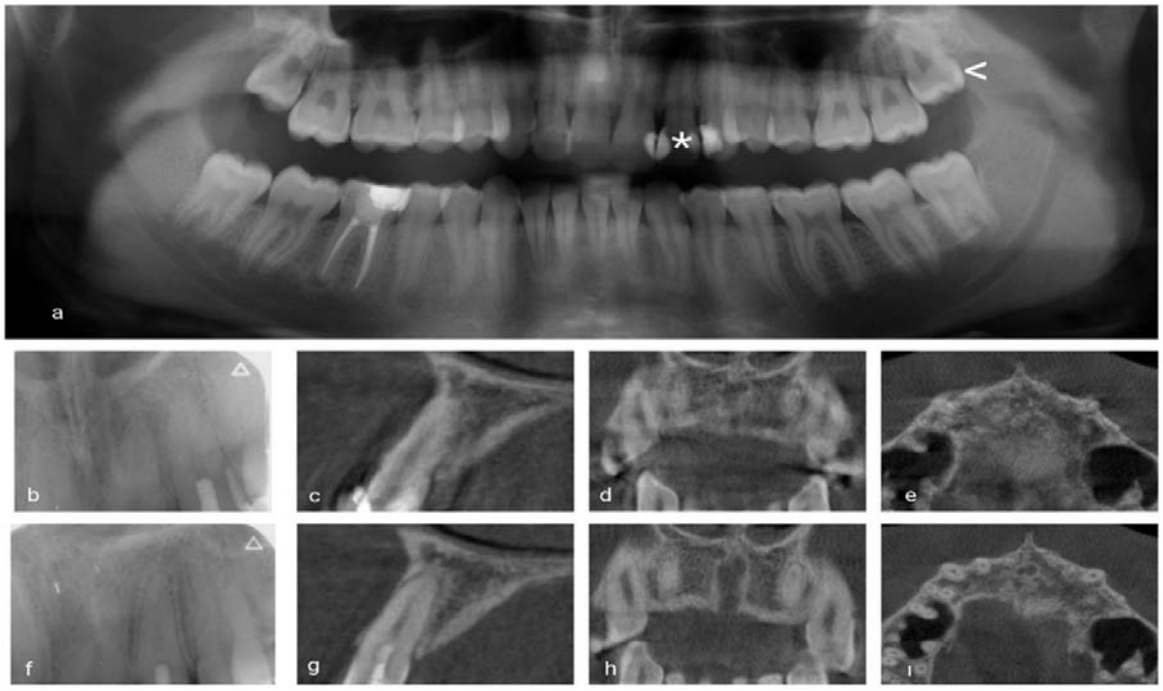

Fig. 4 – Preoperative and 12-month postoperative radiographic and cone-beam computed tomography (CBCT) images of concentrated growth factor-enhanced autologous pulp transplantation (Case 2): a) preoperative panoramic radiograph indicating the recipient tooth and donor third molar; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12-month follow-up showing complete periapical healing. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

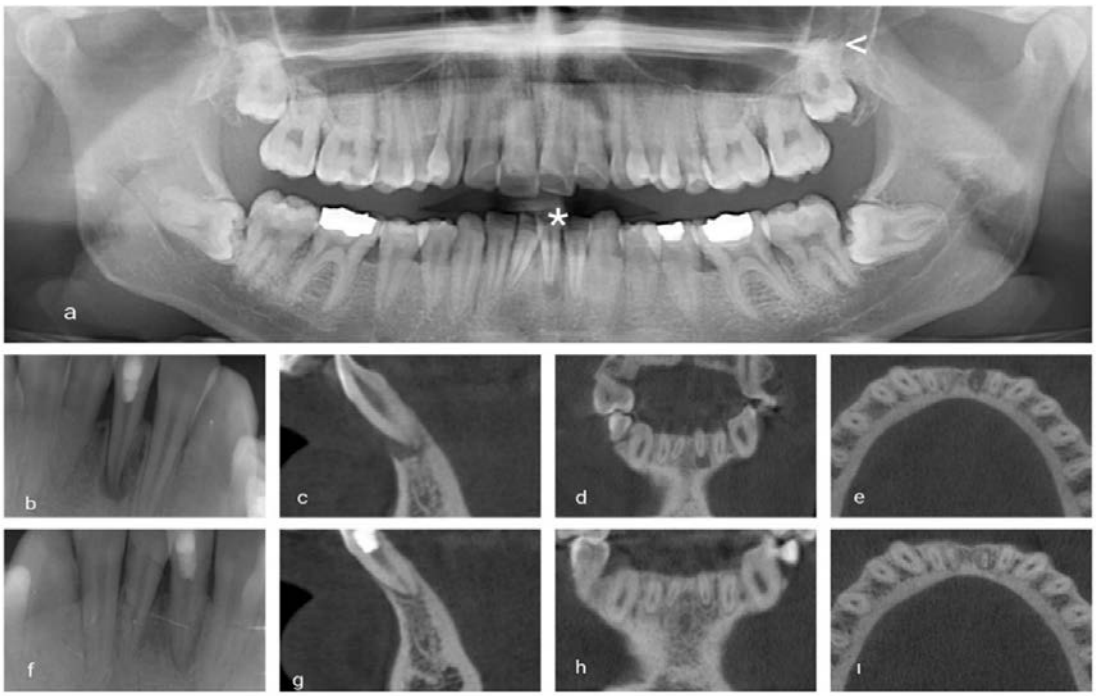

Fig. 5 – Preoperative and 12-month postoperative radiographic and cone-beam computed tomography (CBCT) images of concentrated growth factor-enhanced autologous pulp transplantation (Case 3): a) preoperative panoramic radiograph; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12-month follow-up demonstrating bone regeneration and restoration of apical architecture. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

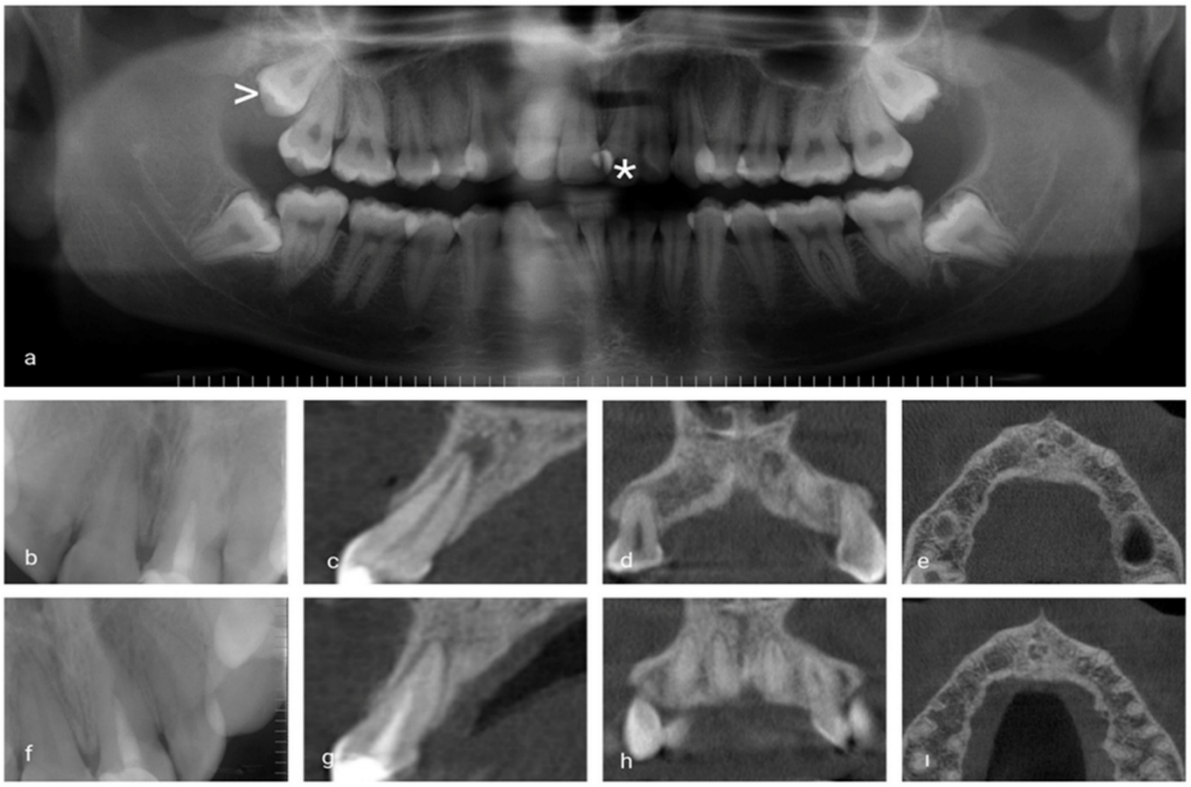

Fig. 6 – Radiographic and cone-beam computed tomography (CBCT) evaluation of concentrated growth factor-enhanced autologous pulp transplantation at baseline and 12-month follow-up (Case 4): a) preoperative panoramic radiograph; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12 months showing resolution of periapical pathology. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

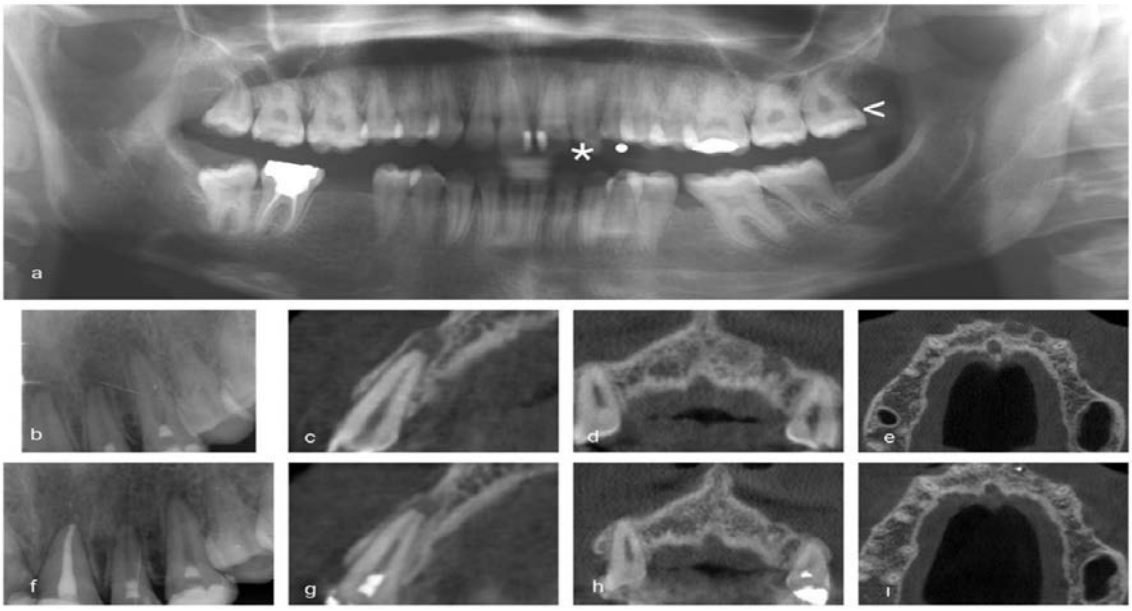

Fig. 7 – Preoperative and 12-month postoperative radiographic and cone-beam computed tomography (CBCT) findings following concentrated growth factor-enhanced autologous pulp transplantation (Case 5): a) preoperative panoramic radiograph identifying the recipient tooth/teeth and donor third molar; b) immediate postoperative periapical radiograph; c–e) preoperative CBCT images (sagittal, coronal, axial); f–i) CBCT images at 12-month follow-up demonstrating periapical healing. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.
***Note*: the symbol "*" denotes the recipient tooth, while "<" indicates the donor third molar.**

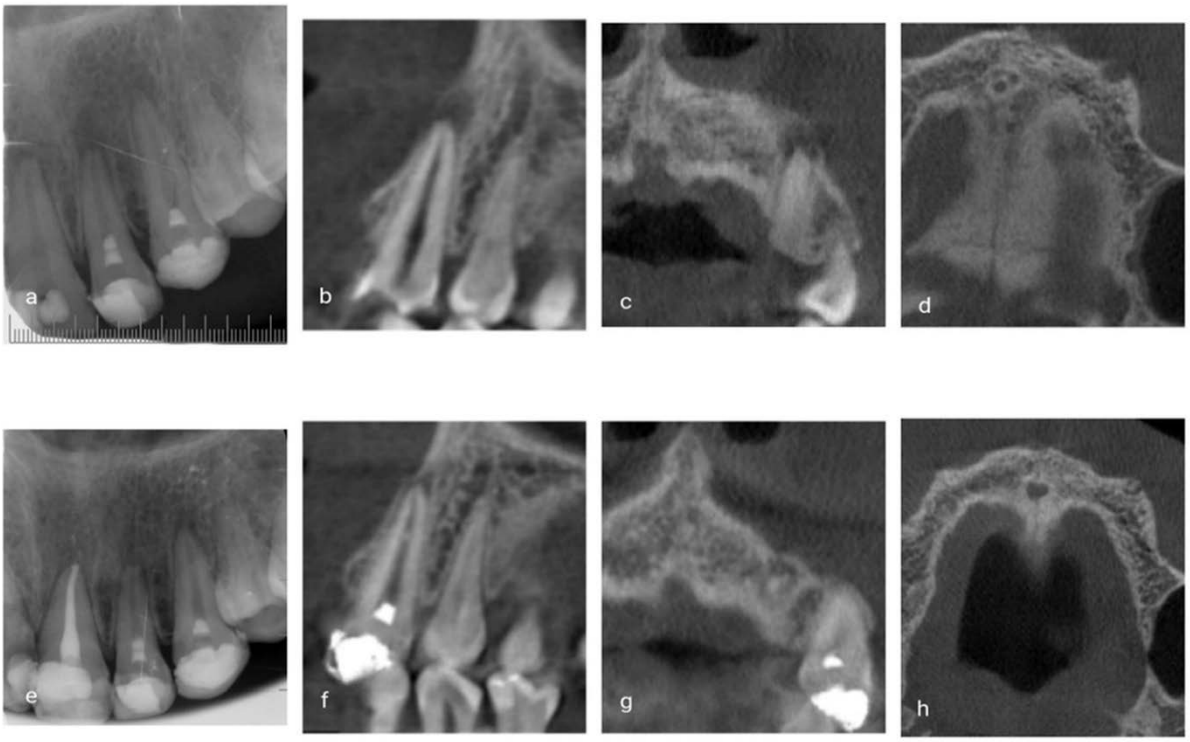

Fig. 8 – Radiographic and cone-beam computed tomography (CBCT) outcomes of concentrated growth factor-enhanced autologous pulp transplantation at baseline and 12-month follow-up (Case 6): a) immediate postoperative periapical radiograph; b–d) preoperative CBCT images (sagittal, coronal, axial); e–h) CBCT images at 12-month follow-up showing complete periapical bone regeneration. CBCT imaging parameters: 90 kV, 8 mA, voxel size 0.2 mm, field of view 8 × 8 cm.

resolution of periapical radiolucency, reformation of trabecular bone, and restoration of apical cortical continuity. Slightly slower healing was noted in two teeth that had undergone additional periodontal and restorative procedures (gingivectomy and composite laminate restoration), although complete healing was ultimately achieved. Representative CBCT images are shown in Figures 3–8.

Discussion

Regenerative endodontic treatment in mature teeth represents an innovative approach in contemporary endodontics, aiming not only to eliminate infection but to biologically restore the vitality and function of the pulp–dentin complex. This treatment signifies a shift from traditional endodontic practices, which typically involve root canal therapy without restoration of the biological sensibility of pulp tissue. When an appropriate regenerative strategy is implemented, the dentin–pulp complex in adult teeth may demonstrate recovery of pulp sensibility responses and other functional signs. However, clinical sensibility testing reflects neural responsiveness and does not by itself confirm histologic pulp regeneration or vitality. The concept of reestablishing functional pulp sensibility, rather than merely obturating the root canal, has, therefore, become a central focus in the current era of biologically based endodontics [1].

Empirical evidence indicates that cell-based approaches are more likely to achieve biologically relevant regeneration of the dentin–pulp complex and restore the tooth's original function compared with cell-free strategies [2]. However, the clinical translation of cell-based therapies remains limited by several critical challenges, including the need for SC isolation and expansion, limited access to facilities compliant with good manufacturing practices, and the high costs of SC culture [3]. Although various cell sources–such as autologous dental pulp cells, allogeneic mesenchymal SCs derived from the umbilical cord, and bone marrow–have been investigated [4], their routine integration into daily dental practice is largely impractical due to logistical, ethical, financial, and regulatory constraints. These limitations highlight the growing demand for simplified, autologous, and chairside-feasible regenerative protocols that preserve biological authenticity while remaining clinically applicable.

In the present study, we aimed to overcome the translational limitations of cell-based regenerative therapies by utilizing the intrinsic regenerative potential of autologous DPSCs without *in vitro* expansion. Unlike protocols that require *ex vivo* cell processing, the SC expansion phase was deliberately omitted. A previous study has reported that DPSCs must be transferred to specialized cell processing facilities and cultured for several days to achieve sufficient proliferation [3], a process that increases contamination risk, complicates clinical application, and substantially elevates

treatment costs. In contrast, in the present study, freshly harvested pulp tissue was directly transplanted into the recipient tooth, which served as a naturally protected biological niche. This environment allows transplanted cells to survive, proliferate, and differentiate under physiological conditions, thereby eliminating the need for *ex vivo* manipulation and enhancing clinical feasibility.

To minimize immunological reactions and transplant rejection, DPSCs were obtained exclusively from the patient's own non-functional, ectopic, or malpositioned wisdom teeth requiring extraction. SCs derived from TMs are considered a suitable source for pulp regeneration due to their pronounced neurogenic differentiation potential and neural crest origin [5, 6]. The autologous nature of this approach reduces the risk of immunogenicity and pathogen transmission while eliminating ethical concerns associated with allogeneic or *ex vivo*–expanded cell sources [7]. These biological and immunological advantages render autologous pulp tissue particularly suitable for direct transplantation.

When combined with a biologically active scaffold, such as CGF, this approach may further enhance cell viability, growth factor release, angiogenesis, and neural regeneration [17]. Although wisdom tooth pulp transplantation provides an immediate, fully autologous regenerative strategy, clinical evidence supporting its efficacy–especially when augmented with biological enhancers such as CGF–remains limited. The present study, therefore, contributes valuable clinical and radiographic data supporting a biologically driven regenerative model that integrates living pulp tissue with endogenous growth factors to promote functional regeneration and periapical healing in mature necrotic teeth.

Current guidelines of the American Association of Endodontists (AAE) advocate the use of low-concentration NaOCl (1.5% NaOCl) for canal disinfection during REPs, primarily to minimize cytotoxic effects on apical SCs [18]. Although higher NaOCl concentrations (5.25%–6.00%) demonstrate superior biofilm removal, they have been shown to exert deleterious effects on SC viability and to reduce the availability of dentin-derived growth factors essential for regeneration [8–10]. The adverse biological impact of NaOCl can be significantly attenuated by subsequent irrigation with 17% EDTA, which not only neutralizes residual hypochlorite but also enhances the release of angiogenic and bioactive molecules such as VEGF and TGF-β1 [11–13]. Importantly, AAE Clinical Considerations are largely extrapolated from *in vitro* studies evaluating cytotoxicity to apical papilla SCs rather than from *in vivo* assessments of antimicrobial efficacy [14]. Moreover, current recommendations are primarily tailored to immature teeth, and standardized irrigation protocols for regenerative procedures in mature teeth are lacking. In the present study, a 2.5% NaOCl solution was selected to balance effective canal disinfection with preservation of biologically active dentin components, particularly in the absence of an apical papilla. Based on the favorable clinical and radiographic outcomes, this concentration appeared sufficient for antimicrobial control without compromising periapical SC viability. Adjunctive irrigation with 17% EDTA was used to mitigate potential cytotoxic effects and promote the release of endogenous growth factors. It should also be considered that in mature teeth with closed apices, the likelihood of irrigant extrusion beyond the apical constriction may be reduced compared with immature teeth. Nevertheless, extrusion risk remains dependent on irrigation technique, needle design, and apical preparation size. Therefore, careful irrigation protocols remain essential to ensure biological safety.

A triple antibiotic paste consisting of cefuroxime, ciprofloxacin, and metronidazole was selected as the intracanal medicament. The exclusion of minocycline from the formulation prevented tooth discoloration, and no discoloration was observed in any case. With this intracanal medication protocol, no additional intracanal medicaments were required throughout treatment.

CGF has emerged as a promising adjunct in regenerative endodontic therapy, particularly for mature teeth with necrotic pulp. CGF is free from bovine thrombin and anticoagulants, addressing limitations associated with platelet-rich plasma and platelet-rich fibrin. It contains high concentrations of growth factors, including TGF-β, PDGF, bone morphogenetic protein, and VEGF. CGF modulates the biological behavior of dental SCs, particularly within inflammatory microenvironments, and has been successfully applied in a limited number of endodontic cases [19].

Preclinical studies have demonstrated that CGF enhances the proliferation, migration, and differentiation of DPSCs, supporting pulp-like tissue formation, neovascularization, and neural elements [20, 21]. The clinical findings of the present study align with these observations, as the use of CGF was associated with encouraging periapical healing findings in this small pilot cohort, along with progressive recovery of pulp sensibility.

In regenerative endodontic treatments, thermal and electric pulp tests are commonly used to assess sensibility. A previous study has reported stable sensibility responses for up to 12 months in mature teeth treated with biologically based regenerative protocols [22]. In CGF-assisted pulp transplantation, positive EPT responses have been reported as early as 3–6 months [23], whereas delayed responses are more frequently observed in protocols without CGF [24]. Consistent with these reports, the present study demonstrated progressive recovery of pulp sensibility, with all treated teeth exhibiting positive EPT responses by the 12-month follow-up. Although EPT demonstrated progressive recovery of sensibility, it should be emphasized that sensibility tests assess neural response rather than direct pulpal blood flow. That being said, they cannot conclusively confirm true pulp vitality. More objective vascular assessment methods, such as laser Doppler flowmetry or pulse oximetry, provide direct evaluation of pulpal microcirculation and may offer more reliable confirmation of revascularization. The absence of vascular vitality testing represents a limitation of the present study and should be addressed in future controlled clinical investigations.

Nevertheless, mature teeth present inherent regenerative limitations due to fully developed root structures and restricted apical access. Although induced apical bleeding has been proposed to facilitate mesenchymal SC

recruitment [25], a previous study indicates that apical enlargement alone does not necessarily improve sensibility outcomes [26]. In the present study, apical foramen sizes ranged between 0.25 mm and 0.30 mm. Yet, positive sensibility responses were achieved in all cases, suggesting that apical diameter alone may not be the primary determinant of regenerative success.

Although apical bleeding was intended in most cases, only minimal bleeding was achieved, possibly due to pre-existing periapical pathology. Unlike previous protocols that supplemented regeneration with blood harvested from extraction sockets [26], no additional blood transfusion was performed. Despite this limitation, complete periapical healing and recovery of pulp sensibility were observed in all cases in this pilot series, suggesting that CGF-enhanced pulp transplantation may be less dependent on extensive blood clot formation and more strongly influenced by viable pulp-derived SCs and sustained growth factor release.

These findings provide preliminary clinical observations suggesting that CGF-enhanced pulp transplantation may represent a potential alternative approach to conventional blood-clot-based regenerative techniques by creating a controlled, growth-factor-rich microenvironment. However, definitive conclusions regarding the nature of the regenerated tissue require histological confirmation. Future histologic and molecular studies are necessary to determine whether the regenerated tissue represents true neurovascular pulp or a reparative pulp-like connective tissue. When interpreted in accordance with the predefined primary outcome and success criteria, the present findings indicate favorable short-term radiographic healing and clinical stability, within the limitations of a small pilot clinical design.

The present evaluation suggests that CGF-enhanced DPSCs may be associated with encouraging short-term periapical healing and recovery of pulp sensibility in this pilot cohort of mature necrotic teeth. By combining two autologous biological components–vital pulp tissue and CGF–this approach may represent a biologically based regenerative strategy that could be applicable in clinical practice, although further controlled studies are required.

Limitations of the study

The findings of this pilot clinical study should be interpreted in light of several limitations. First, the sample size was small (n = 6), which limits the generalizability of the results and prevents statistical inference. Second, the study lacked a control or comparison group, making it difficult to directly evaluate the relative effectiveness of CGF-enhanced pulp transplantation compared with other regenerative endodontic approaches. Third, the follow-up period was limited to 12 months, and longer observation periods are required to determine the long-term stability of the regenerative outcomes. In addition, histological confirmation of the regenerated tissue was not possible in this clinical setting; therefore, the nature of the newly formed tissue could not be definitively verified. Finally, the requirement for donor TM for autologous pulp harvesting may introduce selection bias and limit the broader applicability of this technique. Future controlled clinical studies with larger sample sizes and longer follow-up periods are needed to validate these preliminary findings.

Conclusion

Within the limitations of this pilot clinical study, concentrated growth factor-enhanced autologous pulp transplantation appeared to be a feasible regenerative approach for the management of mature necrotic teeth. The preliminary clinical and radiographic outcomes observed in this small cohort suggest potential for periapical healing and recovery of pulp sensibility responses. However, these findings should be interpreted cautiously due to the limited sample size and absence of a control group. Further well-designed randomized controlled clinical trials with larger patient populations and longer follow-up periods are required to confirm the reproducibility, biological outcomes, and long-term clinical effectiveness of this technique.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Murray PE.* Review of guidance for the selection of regenerative endodontics, apexogenesis, apexification, pulpotomy, and other endodontic treatments for immature permanent teeth. Int Endod J 2023; 56 Suppl 2: 188–99. DOI: 10.1111/iej.13809.
2. *Tirez E, Pedano MS.* Regeneration of the pulp tissue: cell homing versus cell transplantation approach: a systematic review. Materials 2022; 15(23): 8603. DOI: 10.3390/ma15238603.
3. *Nakashima M, Tanaka H.* Pulp regenerative therapy using autologous dental pulp stem cells in a mature tooth with apical periodontitis: a case report. J Endod 2024; 50(2): 189–95. DOI: 10.1016/j.joen.2023.10.015.
4. *Lin L, Huang GTJ, Sigurdsson A, Kahler B.* Clinical cell-based versus cell-free regenerative endodontics: clarification of concept and term. Int Endod J 2021; 54(6): 887–901. DOI: 10.1111/iej.13471.
5. *Ullah I, Park JM, Kang YH, Byun JH, Kim DG, Kim JH,* et al. Transplantation of human dental pulp-derived stem cells or differentiated neuronal cells from human dental pulp-derived stem cells identically enhances regeneration of the injured peripheral nerve. Stem cells Dev 2017; 26(17): 1247–57. DOI: 10.1089/scd.2017.0068.
6. *Orimoto A, Kyakumoto S, Eitsuka T, Nakagawa K, Kiyono T, Fukuda T.* Efficient immortalization of human dental pulp stem cells with expression of cell cycle regulators with the intact chromosomal condition. PLoS One 2020; 15(3): e0229996. DOI: 10.1371/journal.pone.0229996.
7. *Xie Z, Shen Z, Zhan P, Yang J, Huang Q, Huang S,* et al. Functional dental pulp regeneration: basic research and clinical translation. Int J Mol Sci 2021; 22(16): 8991. DOI: 10.3390/ijms22168991.

8. *Jung C, Kim S, Sun T, Cho YB, Song M.* Pulp-dentin regeneration: current approaches and challenges. J Tissue Eng 2019; 10: 2041731418819263. DOI: 10.1177/2041731418819263.
9. *Cai C, Chen X, Li Y, Jiang Q.* Advances in the role of sodium hypochlorite irrigant in chemical preparation of root canal treatment. BioMed Res Int 2023; 2023: 8858283. DOI: 10.1155/2023/8858283.
10. *Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB,* et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014; 40(1): 51–5. DOI: 10.1016/j.joen.2013.07.026.
11. *Galler KM, D'Souza RN, Federlin M, Cavender AC, Hartgerink JD, Hecker S,* et al. Dentin conditioning codetermines cell fate in regenerative endodontics. J Endod 2011; 37(11): 1536–41. DOI: 10.1016/j.joen.2011.08.027.
12. *Ivica A, Deari S, Patcas R, Weber FE, Zehnder M.* Transforming growth factor beta 1 distribution and content in the root dentin of young mature and immature human premolars. J Endod 2020; 46(5): 641–7. DOI: 10.1016/j.joen.2020.01.016.
13. *Mohammadi Z, Shalavi S, Jafarzadeh H.* Ethylenediaminetetraacetic acid in endodontics. Eur J Dent 2013; 7(Suppl 1): S135–S142. DOI: 10.4103/1305-7456.119091.
14. *Wei X, Yang M, Yue L, Huang D, Zhou X, Wang X,* et al. Expert consensus on regenerative endodontic procedures. Int J Oral Sci 2022; 14(1): 55. DOI: 10.1038/s41368-022-00206-z.
15. *Kim JH, Kim Y, Shin SJ, Park JW, Jung IY.* Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod 2010; 36(6): 1086–91. DOI: 10.1016/j.joen.2010.03.031.
16. *Kahler B, Rossi-Fedele G, Chugal N, Lin LM.* An evidence-based review of the efficacy of treatment approaches for immature permanent teeth with pulp necrosis. J Endod 2017; 43(7): 1052–7. DOI: 10.1016/j.joen.2017.03.003.
17. *Jin R, Song G, Chai J, Gou X, Yuan G, Chen Z.* Effects of concentrated growth factor on proliferation, migration, and differentiation of human dental pulp stem cells in vitro. J Tissue Eng 2018; 9: 2041731418817505. DOI: 10.1177/2041731418817505.
18. *American Association of Endodontists.* AAE clinical considerations for a regenerative procedure. Revised 5/18/2021 [Internet]. Chicago: AAE; 2016 [accessed on 2026 April 7]. Available from: https://www.aae.org/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf
19. *Li Z, Liu L, Wang L, Song D.* The effects and potential applications of concentrated growth factor in dentin-pulp complex regeneration. Stem cell Res Ther 2021; 12(1): 357. DOI: 10.1186/s13287-021-02446-y.
20. *Xu F, Qiao L, Zhao Y, Chen W, Hong S, Pan J,* et al. The potential application of concentrated growth factor in pulp regeneration: an in vitro and in vivo study. Stem cell Res Ther 2019; 10(1): 134. DOI: 10.1186/s13287-019-1247-4.
21. *Yu S, Zheng Y, Guo Q, Li W, Ye L, Gao B.* Mechanism of pulp regeneration based on concentrated growth factors regulating cell differentiation. Bioengineering 2023; 10(5): 513. DOI: 10.3390/bioengineering10050513.
22. *Salah T, Hussein W, Abdelkafy H.* Regenerative potential of concentrated growth factor compared to platelet-rich fibrin in treatment of necrotic mature teeth: a randomized clinical trial. BDJ Open 2025; 11(1): 10. DOI: 10.1038/s41405-024-00288-3.
23. *Feitosa VP, Mota MNG, Vieira LV, de Paula DM, Gomes LLR, Solheiro LKR,* et al. Dental pulp autotransplantation: a new modality of endodontic regenerative therapy-follow-up of 3 clinical cases. J Endod 2021; 47(9): 1402–8. DOI: 10.1016/j.joen.2021.06.014.
24. *Cehreli ZC, Unverdi GE, Ballikaya E.* Deciduous tooth pulp autotransplantation for the regenerative endodontic treatment of permanent teeth with pulp necrosis: a case series. J Endod 2022; 48(5): 669–74. DOI: 10.1016/j.joen.2022.01.015.
25. *Chrepa V, Henry MA, Daniel BJ, Diogenes A.* Delivery of apical mesenchymal stem cells into root canals of mature teeth. J Dent Res 2015; 94(12): 1653–9. DOI: 10.1177/0022034515596527.
26. *Kim U, Kim S, Choi SM, Kang MK, Chang I, Kim E.* Regenerative endodontic procedures with minced pulp tissue graft in mature permanent teeth: a clinical study. J Endod 2025; 51(1): 43–53.e2. DOI: 10.1016/j.joen.2024.10.004.

Received on January 14, 2026
Revised on February 23, 2026
Revised on April 3, 2026
Accepted on April 29, 2026
Online First May 2026