Regenerative Therapy 24 (2023) 377–384

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Autologous concentrated growth factor mediated accelerated bone healing in root-end microsurgery: A multicenter randomized clinical trial☆

Yoshio Yahata [a, *], Keisuke Handa [a, b], Naoto Ohkura [c], Motoki Okamoto [d], Jun Ohshima [d], Shusaku Itoh [d], Nobuyuki Kawashima [e], Toshinori Tanaka [a], Nobuya Sato [a], Yuichiro Noiri [c], Mikako Hayashi [d], Takashi Okiji [e], Masahiro Saito [a]

[a] Division of Operative Dentistry, Department of Ecological Dentistry, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
[b] Division of Molecular Biology and Oral Biochemistry, Department of Oral Science, Graduate School of Dentistry, Kanagawa Dental University, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan
[c] Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, 2-5274, Gakkocho-dori, Chuo-ku, Niigata 951-8514, Japan
[d] Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, 1-8 Yamada-Oka, Suita, Osaka 565-0871, Japan
[e] Department of Pulp Biology and Endodontics, Division of Oral Health Sciences, Medical and Dental Sciences Track, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

ARTICLE INFO

Article history:
Received 1 May 2023
Received in revised form
1 August 2023
Accepted 24 August 2023

Keywords:
Root-end microsurgery
Concentrated growth factor
Periapical
Multicenter clinical trial
Regenerative medicine

ABSTRACT

Introduction: Concentrated growth factor (CGF) is a new-generation autologous platelet concentrate that promotes tissue regeneration and has anti-inflammatory properties. This randomized multicenter trial aimed to evaluate the effects of CGF on bone healing in combination with root-end microsurgery.
Methods: Healthy adult patients indicated for root-end microsurgery were randomly assigned to either the CGF or control (no CGF implantation) groups. CGF was implanted into the bone cavity after root-end filling with mineral trioxide aggregate. Clinical and periapical radiographic evaluations were conducted at 1, 3, 6, and 12 months postoperatively, with follow-up cone-beam computed tomography (CBCT) at 6 months. The lesion volume reduction rate was calculated based on data from the preoperative and follow-up CBCT images.
Results: A total of 24 patients were enrolled. The treatment success rate was 91.7% and 83.3% on 12-month periapical radiography and 6-month CBCT, respectively, without a significant difference between the two groups. The lesion volume reduction rate in the CGF group (75.6%) was significantly higher than that in the control (61.0%) group.
Conclusions: Autologous CGF in conjunction with root-end microsurgery accelerated lesion reduction as observed on CBCT. Administering autologous blood products to stimulate healing in addition to removing the source of infection appears to be a promising treatment option for root-end microsurgery.

1. Introduction

Apical periodontitis is an inflammatory disease associated with periapical bone destruction caused by bacterial infection [1]. The pathological process is characterized by granulomatous tissue formation in response to intracanal infection, which may eventually lead to persistent and recurrent inflammation or radicular cysts. Inflammatory response initiated by pathogenic bacteria, their components, and foreign substances released from the infected

Abbreviations: CBCT, Cone-beam computed tomography; CGF, Concentrated growth factor; ITT, Intention-to-treat; PDGF, Platelet-derived growth factor; PRP, Platelet-rich plasma; TGF, Transforming growth factor; VAS, Visual analog pain scale; VEGF, Vascular endothelial growth factor.

☆ This study was registered in the Japan Registry of Clinical Trials (registration number, jRCTc020190025) on March 31, 2020.

* Corresponding author.
E-mail address: yahataendo@tohoku.ac.jp (Y. Yahata).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2023.08.006

root canal stimulate inflammatory cell infiltration and osteoclast recruitment in the periapical tissue, causing bone destruction and accelerated disease progression. Nonsurgical root canal treatment, which reduces the pathogen load in the root canal, has been shown to facilitate healing [2]. However, the complexity of the root canal as well as the host's immune system sometimes interferes with the healing process, resulting in treatment failure with continuous apical bone destruction. Root-end microsurgery utilizing operating microscopes with optimized surgical instrumentation, including ultrasonic retro tips, micromirrors, and biocompatible root-end filling materials, has become a reliable approach with high success rates [3,4].

During root-end microsurgery, the bone cavity created after periapical inflammatory/granulomatous tissue removal is usually left empty, with the assumption that the blood-filled cavity will heal via the body's inherent wound healing capacity. Alternatively, various synthetic materials (including hydroxyapatite, dicalcium phosphate, and calcium phosphate) have been used to promote postsurgical bone repair and regeneration [5–7]. Although synthetic materials are easy to apply, they are associated with risks of bio-incompatibility and immunological rejection. In addition, many synthetic materials are radiopaque, which hinders postsurgical evaluation of bone regeneration.

The healing process in the bone cavity after root-end microsurgery can take several months to years. While most teeth respond quickly and may be classified as healed at the 1-year follow-up, some may take 2–6 years to heal [8,9]. Moreover, large lesions, including those with a preoperative size of >5 mm [10,11], and through-and-through lesions [12,13] have been identified as negative predictors of healing. Guided tissue regeneration techniques utilizing resorbable membranes placed in the bone cavity [14] or platelet-rich plasma (PRP) concentrates [15] in combination with root-end microsurgery have been shown to improve bone regeneration outcomes in large periapical lesions. These findings suggest that considering a treatment strategy that promotes healing is indispensable, especially in the case of a sizable periapical lesion.

Platelets contain a variety of autologous growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-B, vascular endothelial growth factor (VEGF), and insulin-like growth factor, all of which accelerate tissue regeneration and repair [16]. In addition to these, PRP contains anti-inflammatory factors such as interleukin 10 and tissue inhibitors of metalloproteinase-1 [17]. Therefore, PRP therapy is regarded as a promising approach for controlling inflammation [18] and promoting wound healing [19], in cases of bone injury [20], hair regrowth [21], nerve injury [22], and diabetic wounds [23]. Use of platelet concentrates in conjunction with root-end microsurgery has been reported to reduce postoperative pain and improve patient's quality of life [24]. A recent clinical study evaluating PRP in the healing of through-and-through lesions revealed that the PRP group exhibited a significant reduction in lesion volume [15], indicating an improved treatment outcome in such lesions. Concentrated growth factor (CGF), a new-generation autologous platelet concentrate containing equivalent or higher levels of TGF-B1, PDGF-BB, and VEGF than PRP, has been shown to stimulate cell proliferation [25]. It forms a gel-like structure that provides protection from plasmin degradation. CGF is easily obtained by centrifuging intravenous blood of the patient and does not require any synthetics or biomaterials to make a gel; hence, it is free from the risk of cross-contamination [26]. Successful use of CGF in root-end microsurgery has been reported in several studies [27,28]; however, only a few have reported high certainty of evidence. Therefore, it seems reasonable to assume that the adjunctive use of CGF with root-end surgery promotes faster bone healing than conventional root-end microsurgery, although sufficient evidence has yet to be established.

In the present study, we performed a randomized, multicenter trial at four dental university hospitals in Japan to investigate the effects of adjunctive use of CGF in root-end microsurgery. We hypothesized that the use of CGF with root-end microsurgery promotes faster bone healing than conventional root-end microsurgery.

2. Methods

2.1. Study design

The study protocol was approved by the certified review board for regenerative medicine at Tohoku University. A central review board process was chosen for this trial (2018–001). This study was first registered in the Japan Registry of Clinical Trials (registration number, jRCTc020190025) on March 31, 2020. Written informed consent, including for publication of their details, was obtained from all participants prior to enrollment. This multicenter, randomized, controlled prospective clinical trial included patients from four dental universities (Tokyo Medical and Dental University, Osaka University, Niigata University, and Tohoku University), divided into two parallel groups with a 1:1 allocation ratio. We ensured randomization and allocation concealment through a central process at Tohoku University. However, blinding of both surgeons and participants was not possible because of the distinct procedures involved, such as the presence or absence of blood collection or CGF implantation. The conduct and reporting of the trial adhered to the Declaration of Helsinki and the CONSORT guidelines [29].

Experienced endodontists at each participating center selected the patients according to the following inclusion criteria: indicated for root-end microsurgery, noncontributory medical history, platelet count >100,000/mm^3, aged between 20 and 70 years, and lesion size ≥5 mm in diameter on periapical radiography.

The exclusion criteria were as follows: medical history of cancer, age <18 years and >70 years, contraindication for anesthesia or inability to adhere to the trial requirements, infectious diseases (hepatitis B virus, hepatitis C virus, human immunodeficiency virus, or human T-lymphotropic virus-1 infections), vertical or horizontal root fracture, root perforations that were impossible to repair by root-end microsurgery, periodontal probing depths of >5 mm, re-surgery, periapical lesion involving more than two root apices, through-and-through lesions, and lesion size of <5 mm in diameter on periapical radiography.

The data were collected individually at all four dental universities. The study protocol, including patient selection, surgical techniques, and pre- and postoperative measurements, was standardized for all facilities prior to recruitment initiation. Before enrollment, the operator at each center performed periapical radiography and cone-beam computed tomography (CBCT) to confirm participant eligibility. Due to the multicenter nature of the study, various CBCT scanners and fields-of-view were included in the CBCT protocol.

2.2. Root-end microsurgery

All root-end surgeries were conducted in a dedicated operating room with surgical operating microscopes at all the centers. Following local anesthetic administration, a full-thickness buccal flap was reflected to access the periapical lesion. The choice of flap design (intra-sulcular, submarginal, triangular, or trapezoidal) was made by the operator depending on the tooth position, type of

prosthesis, and width of the attached gingiva. In one case with covered cortical bone, the diameter of the cortical bone fenestration was <4 mm; hence, osteotomy was conducted using a round bur. The periapical inflammatory tissue was curetted, and location of the root apex and size of the bone defect were identified. Root-end resection was accomplished approximately 3 mm from the apex, as perpendicular to the long axis of the root as possible. The resected surface was then inspected using micromirrors under high magnification. Thereafter, the root-end cavity preparation was performed using ultrasonic microtips up to a depth of 3 mm in the canal space along the long axis. The root-end was filled with mineral trioxide aggregate (white ProRoot MTA, Dentsply Sirona, Charlotte, NC, USA) mixed with sterile water in a 3:1 powder-to-water ratio according to the manufacturer's instructions.

2.3. CGF preparation

In the CGF group, which received adjunctive implantation of CGF in the bone defect, 10 mL of intravenous blood was drawn by a hospital nurse before the administration of the local anesthetic. Blood was collected in 20-mL glass-coated plastic tubes without an anticoagulant and immediately centrifuged for 15 min at 1600 rpm. After centrifugation, the blood was divided into three layers, with the CGF layer located in the middle, between the red blood cell jelly layer at the bottom and acellular plasma on the top. The clot was removed from the tube, and the lower layer was removed using scissors. The CGF clot was carefully inserted in the bone cavity after root-end filling, and lastly the flap was repositioned and sutured.

2.4. Follow-up and outcome measures

The primary objective was to compare the success rates between the two groups on periapical radiography and CBCT to verify whether CGF accelerates wound healing and bone regeneration, as well as to compare the lesion volume reduction rates using CBCT. As a secondary objective, we assessed the effect of CGF on alleviating postoperative discomfort. Patients were clinically assessed preoperatively and on 1 day, 7 days, 1 month, 3 months, 6 months, and 12 months postoperatively for spontaneous pain, occlusal pain, visual analog pain scale (VAS) score, tenderness on percussion or palpation, and clinical signs (including sinus tract, swelling, and probing depth).

Routine periapical radiography was performed at the 1-month, 3-month, 6-month, and 12-month follow-up visits, while CBCT was performed at the 6-month follow-up visit. Multiple centers participated in this study, each using different scanners and acquisition parameters. Therefore, we set the field of view as small as possible to include the entire lesion and the tooth. Hence, the voxel size used in this study ranged from 0.08 mm to 0.2 mm. The level of healing on the 12-month follow-up periapical radiograph was categorized as complete, incomplete, uncertain, or unsatisfactory, according to the criteria related by Rud et al. [30] and Molven et al. [31]. Regarding the CBCT assessments, the preoperative and 6-month follow-up DICOM format files for each patient were imported to OsiriX MD (Pixmeo, Geneva, Switzerland). Thereafter, buccopalatal and mesiodistal images that aligned with the root canal axis and included the apical area were obtained from each scan, using the two-dimensional multiplanar reconstruction mode in the software. The ideal image for assessment was determined by an experienced endodontist who was not involved in the intervention or radiographic evaluation of the study. Assessment of healing on the CBCT scan was performed using the modified Penn three-dimensional criteria according to Safi et al. [32]. The outcomes were classified as complete, limited, or unsatisfactory healing.

Two calibrated examiners, who were experienced endodontists (TT and NS), assessed all the periapical radiographs and CBCT images independently. These examiners were not involved in the interventions and were blinded to the time of follow-up and application of CGF. Disagreements were resolved through discussion until a consensus was reached. Successful healing was determined by the absence of clinical symptoms with categorization of complete or incomplete healing on periapical radiographs and complete or limited healing on CBCT images. Failure was defined as an assessment that could not be classified as successful healing. Additionally, any dropouts or excluded cases after randomization were considered as failures.

The lesion volume reduction rate was measured using preoperative and 6-month postoperative CBCT images and the Amira image analysis software (Thermo Fisher Scientific, Waltham, MA, USA). The lesion areas of all horizontal cross sections were extracted from the DICOM images and volume was calculated by three-dimensional reconstruction of the lesion from the extracted lesion area of each cross section and the image thickness. The volume of the lesion was calculated preoperatively and 6 months postoperatively using CBCT images. The volume reduction rate was calculated as follows:

$$\textit{Lesion volume reduction rate}\ (\%) = \frac{(\textit{preoperative volume} - \textit{postoperative volume}) \times 100}{\textit{preoperative volume}}$$

2.5. Statistical analysis

The sample size was calculated based on the available similar research at the time of study protocol development [33], which indicated the lesion volume reduction rate with the control group was 33%. The sample size was determined to estimate an effect size of 1.4 with α error of 0.05 and a power of 0.80. The minimum sample size was calculated to be 20 ($n = 10$ in each group). To prevent an imbalance in the number of cases between the four centers, we recruited a total of 24 patients ($n = 6$ per center). After the patients fulfilled the selection criteria and provided informed consent at each trial center, the operator sent a request form to the primary center (Tohoku University). Randomization concealment was warranted because randomization was conducted centrally after the enrollment phase. At the primary center, a person who was not the operator or examiner assigned the participants randomly to either the CGF or control group using a computer-based stratified blocked randomization method. Since the operative procedures involved blood withdrawal, centrifugation, and CGF implantation, which required visualization, neither the operator nor the patient could be blinded.

The chi-square test was used to analyze the variability in enrollment between the CGF and control groups. A weighted kappa coefficient was used to assess the interobserver agreement of periapical radiographs and CBCT images. Comparison of the success rates between the groups at 12 months with periapical radiography and at 6 months with CBCT, in conjunction with the clinical symptoms, was analyzed using the chi-square test based on the

intention-to-treat (ITT) population. A per-protocol analysis was conducted since the lesion volume reduction rate and VAS score were continuous variables. The Shapiro–Wilk test showed that the dataset of the lesion volume reduction rate followed a normal distribution; hence, Student's *t*-test was used to test differences between the two groups. On the other hand, Mann–Whitney *U* test was used to compare the VAS values because the dataset departed from normality. The significance level was set at alpha = 0.05.

3. Results

3.1. Study population

This study enrolled 24 patients following an initial assessment according to the inclusion and exclusion criteria (Fig. 1). During investigation of the resected root surface, a vertical fracture was detected in one patient from the CGF group (Fig. 1); therefore, follow-up was discontinued for this patient. Since the fracture was identified after randomization, this case was classified as a failure and included in the ITT analysis.

Table 1 shows the demographic distribution of the participants. There were no significant differences between the groups in terms of sex, age, tooth position, jaw position, presence of systemic disease, or preoperative symptoms ($P > 0.05$, chi-square test).

3.2. Outcome assessment

The total success rate assessed using periapical radiography at 12 months was 91.7% (Table 2). Although no significant difference was observed in the success rates between the CGF and control groups evaluated by periapical radiography (83.3% vs. 100%; $P = 0.1396$, chi-square test) and CBCT (66.7% vs. 91.7%; $P = 0.1316$, chi-square test) (Table 3), the lesion volume reduction rate was 75.6% in the CGF group and 61.0% in the control group, with a significantly higher reduction rate in the former ($P = 0.0475$, Student's *t*-test) (Fig. 2). Interobserver agreement was good for both periapical radiographs ($K = 0.73$, $P < 0.001$, weighted kappa) and CBCT images ($K = 0.79$, $P < 0.001$, weighted kappa).

A typical case of success with respect to healing in the CGF group is shown in Fig. 3. This case was classified as complete healing on periapical radiography at 12 months and on CBCT at 6 months, both of which showed increased radiopacity in the periapical area. A typical case of failed outcome in the CGF group is shown in Fig. 4. With regard to the VAS scores, there was no significant difference between the two groups preoperatively and at all postoperative appointments ($P > 0.05$, Mann–Whitney *U* test).

4. Discussion

Preoperative radiographic size of a periapical lesion is regarded as one of the contributing factors of extended healing after root-end surgery. Lesions >5 mm in diameter or through-and-through lesions (lesions penetrating the cortical bone on both the labial and palatal sides) are negative predictors of root-end surgery outcomes [10–13]. A previous report showed that PRP effectively reduced the lesion volume after 1 year in through-and-through lesions [15]. In the present study, it was found that CGF contributed to accelerated alveolar bone healing by reducing the lesion volume, suggesting that CGF is a suitable adjunctive material for root-end microsurgery.

The main objective of endodontic treatment is to remove the source of infection. In most cases, a favorable prognosis with the maintenance of tooth function can be anticipated by practicing the established surgical and nonsurgical procedures. Traditionally, eradication of bacteria from the root canal has been emphasized in management of periapical lesions, while little attention has been

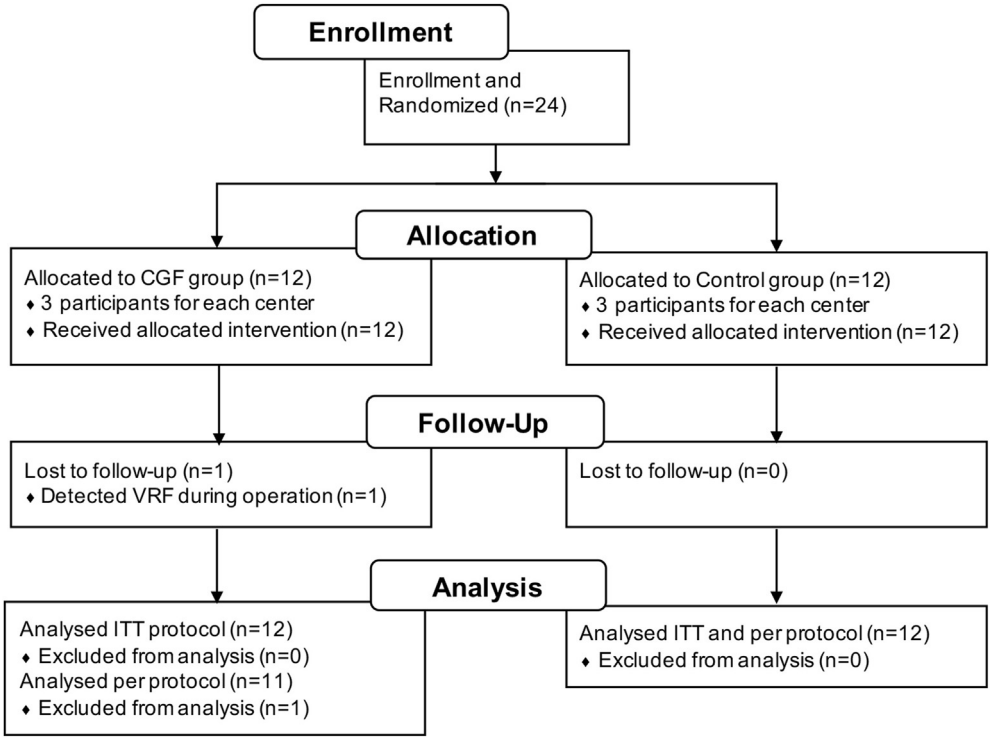

Fig. 1. Patient selection. CONSORT flow chart outlining selection of the study participants.

Table 1
Demographic distribution of cases at base line in the intention-to-treat population.

Characteristic	Total (n = 24)		CGF group (n = 12)		Control group (n = 12)		*P* value (chi-square)
	n	(%)	n	(%)	n	(%)	
Sex							
Male	8	33.3	3	25.0	5	41.7	0.3865
Female	16	66.7	9	75.0	7	58.3	
Age, years							
30–50	11	45.8	7	58.3	4	33.3	0.2191
>50	13	54.2	5	41.7	8	66.7	
Tooth position							
Anterior	15	62.5	9	75.0	6	50.0	0.2059
Posterior	9	37.5	3	25.0	6	50.0	
Jaw							
Maxilla	23	95.8	11	91.7	12	100.0	0.3070
Mandible	1	4.2	1	8.3	0	0.0	
Systemic disease							
Present	4	16.7	2	16.7	2	16.7	1.0000
Absent	20	83.3	10	83.3	10	83.3	
Preoperative symptoms							
Spontaneous pain							
Present	6	25.0	3	25.0	3	25.0	1.0000
Absent	18	75.0	9	75.0	9	75.0	
Occlusal pain							
Present	7	29.2	4	33.3	3	25.0	0.6534
Absent	17	70.8	8	66.7	9	75.0	
Tender to percussion							
Present	10	41.7	6	50.0	4	33.3	0.4076
Absent	14	58.3	6	50.0	8	66.7	
Tender to palpation							
Present	12	50.0	6	50.0	6	50.0	1.0000
Absent	12	50.0	6	50.0	6	50.0	
Periodontal pocket							
<3 mm	20	83.3	10	83.3	10	83.3	1.0000
>4 mm	4	16.7	2	16.7	2	16.7	
Sinus tract							
Present	8	33.3	3	25.0	5	41.7	0.3865
Absent	16	66.7	9	75.0	7	58.3	

CGF, concentrated growth factor.

paid to assessing the bone healing capacity of individual patients. Inflammation caused by microbial infection and surgical intervention can lead to the production of proinflammatory cytokines that interfere with bone healing [34]. Thus, regulation of inflammation is one of the key factors for successful bone healing. We did not measure the concentration of growth factors in individual CGF clots obtained from patients in this study. However, a study that used the same methodology as that used in the current study reported that the CGF clots were rich sources of growth factor components effective for anti-inflammation, such as TGF-B1, PDGF-BB, and VEGF [25]. Recent reports have indicated that CGF facilitates

Table 3
Summary of treatment outcome by preoperative and 6-month postoperative cone-beam computed tomography assessment in the intention-to-treat population. There were no significant differences between the two groups (chi-square, $P > 0.05$).

Outcome	Total (n = 24)		CGF group (n = 12		Control group (n = 12)		
	n	(%)	n	(%)	n	(%)	
CBCT assessmentrowhead							
Complete healing	2	8.3	2	16.7	0	0.0	
Limited healing	17	75.0	6	50.0	11	91.7	
Unsatisfactory healing	4	16.7	3	25.0	1	8.3	
Unknown (excluded after allocation)	1	4.2	1	8.3	0	0.0	
							P value (chi-square)
Success	19	79.2	8	66.7	11	91.7	0.1316
Failure	5	20.8	4	23.3	1	8.3	

CBCT, cone-beam computed tomography; CGF, concentrated growth factor.

M2 macrophage polarization, resulting in suppression of proinflammatory cytokines [35,36]. Furthermore, CGF has been proven to be critical to wound healing by serving as a wound-healing matrix, delivering various growth factors that stimulate osteogenic cell migratory pathways [37]. Adding CGF to human dental pulp stem cells causes extensive upregulation of bone-related genes such as DSPP, DMP1, OPN, RUNX2, and OCN, significantly increasing the mineralized areas shown by alizarin red staining [36]. The consolidated findings of the current and other reports suggest that both anti-inflammatory effects and growth factors that cause osteoblast recruitment contribute towards accelerated bone healing when CGF is implanted. In the current study, CGF implantation did not influence postoperative pain intensity, which was consistent with the results of a previous study [38]. Hence, our data indicated that the adjunctive use of CGF during root-end microsurgery directly stimulated active bone healing without increasing postoperative pain.

A high prevalence of periapical lesions has been reported among patients with systemic diseases [39,40]. Patients with immune disorders present sustained inflammation and delayed wound healing. CGF is known to promote healing in chronic ulcerative wounds and wounds in patients with systemic diseases such as diabetes [23]. Thus, we propose using CGF as an adjunct during root-end microsurgery to stimulate active bone healing, especially in patients with inferior healing capacity due to systemic diseases.

Table 2
Summary of treatment outcome by preoperative and 12-month postoperative radiographic assessment in the intention-to-treat population. There were no significant differences between the two groups (chi-square, $P > 0.05$).

Outcome	Total (n = 24)		CGF group (n = 12)		Control group (n = 12)		
	n	(%)	n	(%)	n	(%)	
Radiographic assessmentrowhead							
Complete healing	18	75.0	8	66.7	10	83.3	
Incomplete healing	4	16.7	2	16.7	2	16.7	
Uncertain healing	1	4.2	1	8.3	0	0.0	
Unsatisfactory healing	0	0.0	0	0.0	0	0.0	
Unknown (excluded after randomization)	1	4.2	1	8.3	0	0.0	
							P value (chi-square)
Success	22	91.7	10	83.3	12	100.0	0.1396
Failure	2	8.3	2	16.7	0	0.0	

CGF, concentrated growth factor.

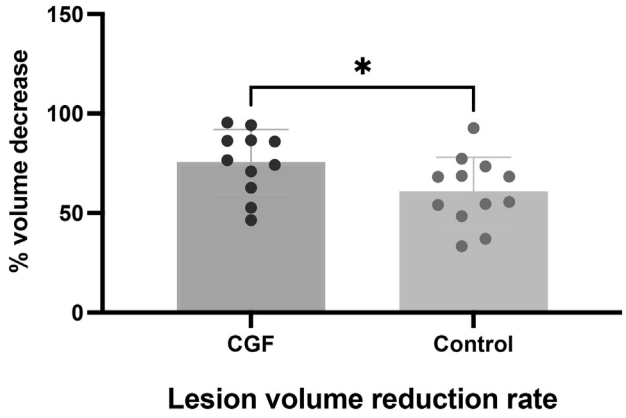

Fig. 2. Reduction rate of lesion volume. A significant difference in lesion volume reduction rate is noted between the CGF and control groups as the per-protocol set (Shapiro–Wilk test, $P > 0.05$; Student's t-test, $P = 0.0475$). Each dot represents the reduction rate of each case. CGF, concentrated growth factor.

In the present study, one case of intraoperative vertical root fracture was encountered in the CGF group after randomization. In most previous clinical trials reporting the outcome of root-end microsurgery, such cases were categorized as dropouts and excluded from the analysis. However, in this study, the ITT analysis was conducted after classifying the singular dropout case as a failure on healing evaluation by imaging, to follow the ITT principle of including all randomized participants assigned to the randomized treatment, regardless of noncompliance, protocol deviations, withdrawal, or any event occurring after randomization [41]. The ITT protocol is considered more conservative than the per-protocol for avoiding overoptimistic estimates of the efficacy of treatment regimens [42]. Furthermore, the CONSORT guidelines have addressed the importance of ITT analysis in improving the quality of randomized clinical trials [29]. Therefore, ITT analysis can be regarded as an appropriate method to evaluate novel treatment procedures using CGF.

This study was a multicenter study conducted at four university hospitals in Japan. The external validity of the results of a multicenter study is higher than that of a single-center study. In the present study, there was no intercenter variability in the success rate of root-end microsurgery or the efficacy of CGF due to the difference in the success rates among the centers. Therefore, this study can form the basis for future large-scale clinical multicenter studies.

CBCT evaluation in the present study confirmed that CGF accelerated bone healing, suggesting that CGF is a suitable material for application in root-end microsurgery. However, the number of cases in this study was relatively small (n = 24; 12 cases in each group). Future studies with a larger sample size are warranted to determine the robustness of our findings. While CGF showed accelerated bone healing in the reconstructed CBCT images, the evaluator-assessed criteria did not show outcome differences between periapical radiography and CBCT images. The uncertainty of visual healing findings on imaging may limit its usefulness in determining treatment success. In our study, CBCT was conducted 6 months postoperatively, which can be considered as an early-stage evaluation. We believe that early-stage evaluation during the healing process was essential to verify our hypothesis that CGF-

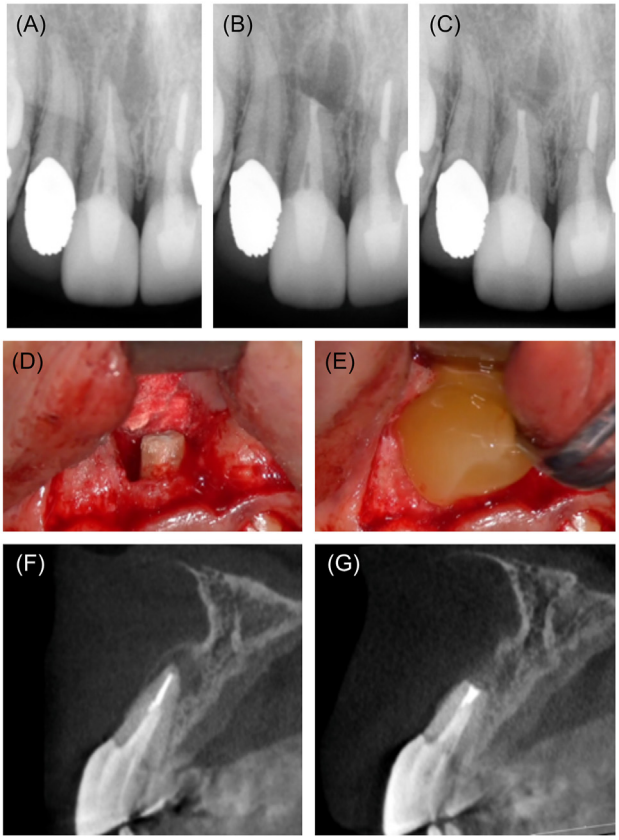

Fig. 3. A typical case of complete healing in the CGF group The preoperative radiograph shows a circular radiolucent area around the root apex of the right maxillary central incisor (A). The postoperative radiograph reveals the resected root apex and root-end filling material inside the residual root canal (B). The radiograph captured 12 months after root-end microsurgery confirms a decreased periapical radiolucent area (C). Intraoperative photographs were taken before (D) and during (E) concentrated growth factor implantation into the bone cavity. The preoperative cone-beam computed tomography sagittal section image shows a radiolucent area around the root apex (F). The 6-month postoperative sagittal section cone-beam computed tomography image demonstrates the formation of radiopaque structures in the preoperative radiolucent area, including the site of the apical resection, suggesting bone regeneration (G).

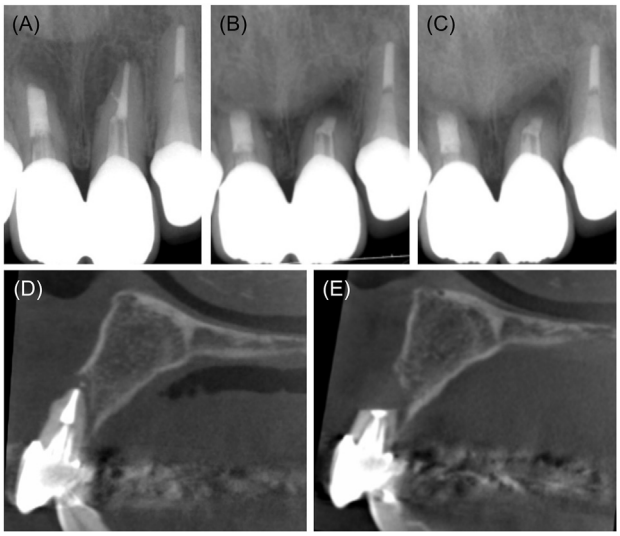

Fig. 4. A typical case of uncertain healing in the CGF group. The preoperative radiograph shows a root canal filling around the apex and lateral canal as well as a radiolucent area surrounding the apex of the left maxillary central incisor (A). The immediately postoperative radiograph shows the resected root apex and lateral canal (B). The 12-month postoperative radiograph indicates no significant changes in radiolucency of the periapical area (C). A comparison of the preoperative (D) and postoperative (E) sagittal cone-beam computed tomography images reveal the absence of bone healing in the surrounding alveolar boneCGF, concentrated growth factor.

mediated healing promotes accelerated alveolar bone regeneration. Additional clinical trials with long-term outcome assessments are warranted to clarify the effectiveness of CGF.

5. Conclusions

The healing effect of autologous CGF in conjunction with root-end microsurgery showed accelerated lesion reduction on CBCT. Administering autologous blood products in addition to removing the infection source through standard root-end microsurgery procedure seems to be a promising treatment option to stimulate healing, particularly in patients with impaired healing due to systemic diseases.

Author contributions

Y.Y., K.H., and M.S. conceptualized and designed the study. K.H., N.O., M.O., J.O., and N.K. collected experimental data. Y.Y., T.T., and N.S. contributed to the analysis and interpretation of the data. Y.Y. drafted the initial manuscript. S.I., Y.N., M.H., T.O., and M.S. supervised conduction of the study and reviewed the manuscript. All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare no conflicts of interests in relation to this study.

Acknowledgment

This study was funded by the Japanese Association for Dental Science, Sponsored Research 2017.

References

[1] Siqueira Jr JF, Rôças IN. Present status and future directions: microbiology of endodontic infections. Int Endod J 2022;55(Suppl 3):512–30.
[2] Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature - part 1. Effects of study characteristics on probability of success. Int Endod J 2007;40:921–39.
[3] Setzer FC, Shah SB, Kohli MR, Karabucak B, Kim S. Outcome of endodontic surgery: a meta-analysis of the literature–part 1: comparison of traditional root-end surgery and endodontic microsurgery. J Endod 2010;36:1757–65.
[4] Tsesis I, Rosen E, Taschieri S, Telishevsky Strauss Y, Ceresoli V, Del Fabbro M. Outcomes of surgical endodontic treatment performed by a modern technique: an updated meta-analysis of the literature. J Endod 2013;39:332–9.
[5] Naylor J, Mines P, Anderson A, Kwon D. The use of guided tissue regeneration techniques among endodontists: a web-based survey. J Endod 2011;37:1495–8.
[6] Bernabé PF, Gomes-Filho JE, Cintra LT, Moretto MJ, Lodi CS, Nery MJ, et al. Histologic evaluation of the use of membrane, bone graft, and MTA in apical surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:309–14.
[7] Pecora G, De Leonardis D, Ibrahim N, Bovi M, Cornelini R. The use of calcium sulphate in the surgical treatment of a 'through and through' periradicular lesion. Int Endod J 2001;34:189–97.
[8] Kruse C, Spin-Neto R, Christiansen R, Wenzel A, Kirkevang LL. Periapical bone healing after apicectomy with and without retrograde root filling with mineral trioxide aggregate: a 6-year follow-up of a randomized controlled trial. J Endod 2016;42:533–7.
[9] Kvist T, Reit C. Results of endodontic retreatment: a randomized clinical study comparing surgical and nonsurgical procedures. J Endod 1999;25:814–7.
[10] von Arx T, Jensen SS, Hänni S, Friedman S. Five-year longitudinal assessment of the prognosis of apical microsurgery. J Endod 2012;38:570–9.
[11] Shinbori N, Grama AM, Patel Y, Woodmansey K, He J. Clinical outcome of endodontic microsurgery that uses EndoSequence BC root repair material as the root-end filling material. J Endod 2015;41:607–12.
[12] Skoglund A, Persson G. A follow-up study of apicoectomized teeth with total loss of the buccal bone plate. Oral Surg Oral Med Oral Pathol 1985;59:78–81.
[13] Hirsch JM, Ahlström U, Henrikson PA, Heyden G, Peterson LE. Periapical surgery. Int J Oral Surg 1979;8:173–85.
[14] Taschieri S, Corbella S, Tsesis I, Bortolin M, Del Fabbro M. Effect of guided tissue regeneration on the outcome of surgical endodontic treatment of through-and-through lesions: a retrospective study at 4-year follow-up. Oral Maxillofac Surg 2011;15:153–9.
[15] Dhamija R, Tewari S, Sangwan P, Duhan J, Mittal S. Impact of platelet-rich plasma in the healing of through-and-through periapical lesions using 2-dimensional and 3-dimensional evaluation: a randomized controlled trial. J Endod 2020;46:1167–84.
[16] Kawase T. Platelet-rich plasma and its derivatives as promising bioactive materials for regenerative medicine: basic principles and concepts underlying recent advances. Odontology 2015;103:126–35.
[17] Moussa M, Lajeunesse D, Hilal G, El Atat O, Haykal G, Serhal R, et al. Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage. Exp Cell Res 2017;352:146–56.
[18] El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, et al. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol 2007;78:661–9.
[19] Roubelakis MG, Trohatou O, Roubelakis A, Mili E, Kalaitzopoulos I, Papazoglou G, et al. Platelet-rich plasma (PRP) promotes fetal mesenchymal stem/stromal cell migration and wound healing process. Stem Cell Rev Rep 2014;10:417–28.
[20] Malhotra A, Pelletier MH, Yu Y, Walsh WR. Can platelet-rich plasma (PRP) improve bone healing? A comparison between the theory and experimental outcomes. Arch Orthop Trauma Surg 2013;133:153–65.
[21] Gentile P, Garcovich S, Bielli A, Scioli MG, Orlandi A, Cervelli V. The effect of platelet-rich plasma in hair regrowth: a randomized placebo-controlled trial. Stem Cells Transl Med 2015;4:1317–23.
[22] Yu W, Wang J, Yin J. Platelet-rich plasma: a promising product for treatment of peripheral nerve regeneration after nerve injury. Int J Neurosci 2011;121:176–80.
[23] Kao CH. Use of concentrate growth factors gel or membrane in chronic wound healing: description of 18 cases. Int Wound J 2020;17:158–66.
[24] Del Fabbro M, Ceresoli V, Lolato A, Taschieri S. Effect of platelet concentrate on quality of life after periradicular surgery: a randomized clinical study. J Endod 2012;38:733–9.
[25] Masuki H, Okudera T, Watanebe T, Suzuki M, Nishiyama K, Okudera H, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). Int. J. Implant Dent. 2016;2:19.
[26] Rodella LF, Favero G, Boninsegna R, Buffoli B, Labanca M, Scarì G, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Tech 2011;74:772–7.
[27] Sureshbabu NM, Ranganath A, Jacob B. Concentrated growth factor - surgical management of large periapical lesion using a novel platelet concentrate in combination with bone graft. Ann Maxillofac Surg 2020;10:246–50.
[28] Sureshbabu NM, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: a report of two cases. Case Rep Dent 2019;2019:7046203.
[29] Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c332.
[30] Rud J, Andreasen JO, Jensen JE. Radiographic criteria for the assessment of healing after endodontic surgery. Int J Oral Surg 1972;1:195–214.
[31] Molven O, Halse A, Grung B. Observer strategy and the radiographic classification of healing after endodontic surgery. Int J Oral Maxillofac Surg 1987;16:432–9.
[32] Safi C, Kohli MR, Kratchman SI, Setzer FC, Karabucak B. Outcome of endodontic microsurgery using mineral trioxide aggregate or root repair material as root-end filling material: a randomized controlled trial with cone-beam computed tomographic evaluation. J Endod 2019;45:831–9.
[33] Ahmed GM, Nageh M, Elbaz A, Saif N, Moustafa OH. CBCT volumetric evaluation of bone healing after endodontic microsurgery using platelet-rich fibrin (PRF). Endod Pract Today 2018;12:241–8.
[34] Maruyama M, Rhee C, Utsunomiya T, Zhang N, Ueno M, Yao Z, et al. Modulation of the inflammatory response and bone healing. Front Endocrinol 2020;11:386.
[35] Luo H, Liu W, Zhou Y, Jiang X, Liu Y, Yang Q, et al. Concentrated growth factor regulates the macrophage-mediated immune response. Regen Biomater 2021;8:rbab049.
[36] 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;10:134.
[37] Honda H, Tamai N, Naka N, Yoshikawa H, Myoui A. Bone tissue engineering with bone marrow-derived stromal cells integrated with concentrated growth factor in Rattus norvegicus calvaria defect model. J Artif Organs 2013;16:305–15.
[38] Meschi N, Fieuws S, Vanhoenacker A, Strijbos O, Van der Veken D, Politis C, et al. Root-end surgery with leucocyte- and platelet-rich fibrin and an occlusive membrane: a randomized controlled clinical trial on patients' quality of life. Clin Oral Invest 2018;22:2401–11.
[39] Poyato-Borrego M, Segura-Sampedro JJ, Martín-González J, Torres-Domínguez Y, Velasco-Ortega E, Segura-Egea JJ. High prevalence of apical

periodontitis in patients with inflammatory bowel disease: an age- and gender- matched case-control study. Inflamm Bowel Dis 2020;26:273–9.
[40] Khalighinejad N, Aminoshariae MR, Aminoshariae A, Kulild JC, Mickel A, Fouad AF. Association between systemic diseases and apical periodontitis. J Endod 2016;42:1427–34.
[41] Gupta SK. Intention-to-treat concept: a review. Perspect Clin Res 2011;2: 109–12.
[42] White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ 2011; 342:d40.