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ORIGINAL ARTICLE/ARTICOLO ORIGINALE

Application of platelet-rich fibrin in endodontic surgery: a pilot study

Applicazione del platelet-rich fibrin in endodonzia chirurgica: studio pilota

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KEYWORDS
Endodontic surgery;
Healing;
Platelet-rich fibrin;
Postoperative pain;
Swelling.

Abstract
Aim: To assess preliminarily the potential benefits of the use of the platelet-rich fibrin (PRF) in modern endodontic surgical procedures in terms of radiographic healing acceleration and postoperative discomfort reduction.
Methodology: Eleven patients with chronic apical periodontitis were randomly assigned to either the PRF ($n = 7$) or the control group ($n = 4$). Postoperative swelling and pain were assessed with a questionnaire. Radiographic healing was scored according to Molven's scale up to a period of one year. Data were statistically analyzed with non-parametric tests.
Results: In the PRF group the patients experienced less pain in the 2—6 h postoperatively as well as oedema, which never exceeded the moderate intraoral swelling. Radiographic healing was detectable earlier in the PRF group, with the majority of cases scored as complete healing after 2—3 months.
Conclusions: The adjunctive use of PRF might promote the acceleration of the radiographic healing and reduce the postoperative discomfort.

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PAROLE CHIAVE
Endodonzia chirurgica;
Guarigione;
Platelet-rich fibrin;
Dolore postoperatorio;
Gonfiore.

Riassunto
Obiettivi: Valutare preliminarmente i potenziali benefici dell'uso del platelet-rich fibrin (PRF) nella moderna endodonzia chirurgica in termini di accelerazione della guarigione radiografica e riduzione del discomfort postoperatorio.
Materiali e metodi: Undici pazienti con paradentite periapicale cronica sono stati assegnati casualmente al gruppo PRF ($n = 7$) o al gruppo controllo ($n = 4$). Gonfiore e dolore postoperatori sono stati valutati con un questionario. Nell'arco di un anno di osservazione è stato assegnato un punteggio alla guarigione radiografica secondo la scala di Molven. I dati sono stati analizzati statisticamente con test non parametrici.
Risultati: I pazienti del gruppo PRF hanno provato meno dolore nelle 2—6 ore postoperatorie e sviluppato minor edema, che era sempre limitato e intraorale. Nel gruppo PRF la guarigione radiografica era individuabile precocemente, con la maggioranza dei casi classificata come guarigione completa dopo 2—3 mesi.
Conclusioni: L'uso aggiuntivo del PRF sembra promuovere l'accelerazione della guarigione radiografica e ridurre il discomfort postoperatorio.

Introduction

Untreated pulp tissue necrosis may lead to periapical periodontitis, which represents a response of the bone around the apex to restrain the local infective offence. Periapical healing can be achieved by root canal treatment, whose purpose is to remove bacteria and remnants of infected tissue by shaping, cleaning and filling with an inert material the entire root canal system.[1] The main cause of unsuccessful periapical healing after primary endodontic therapy is the persistence of bacteria and infected tissue in the endodontic space[2] even after orthograde endodontic treatment and retreatment; in such cases, the last resort to maintain the tooth is represented by apical surgery.[3] In the choice between orthograde re-treatment and surgical approach, the latter has to be preferred when the root canal filling is adequate, but symptoms are persisting, when re-treatment involves high risk procedures or long posts are present in the root canal.[4]

Improvement in technical instruments and in surgical techniques might enhance the outcome of endodontic surgery.[5] In fact, the employment of microsurgical techniques and modern obturation materials raised the success rates after root-end resection and retrograde filling to about 80—90%.[6,7] In order to induce bone regeneration and soft tissues healing after oral surgery, the local application of hormones, grow factors and plasma derivates has been advocated.[8] Platelet-rich plasma (PRP), bone morphogenic proteins (BMPs), platelet-derived growth factor (PDGF), parathyroid hormone (PTH), and enamel matrix proteins (EMD) have been locally applied to promote the healing potential of the surgical site.[8] Nevertheless, the effectiveness of their application in endodontic surgery is still questionable and the advantages they provide to both surgeon and patient have been reported to be moderate and remain still controversial.[9—14]

It has been advocated that Platelet-rich Fibrin (PRF) can be considered a healing biomaterial because it is constituted by a fibrin network in which platelets, leukocytes, cytokines and stem cells are enmeshed.[15] Moreover, the platelets in the PRF network are capable of slowly releasing platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF),[16,17] even up to one week.[18] The osteogenic potential of these molecules has been already demonstrated.[19,20] PRF can be thought as a grow factor reservoir that can be employed without exposing the patient to any immunogenicity or infection risk,[21] because it is entirely composed of nothing but the patient's blood. The application of such a specific biomaterial to endodontic surgery has already been described in some recent case reports[22—24] and a randomized clinical trial in the specifical field of the treatment of apicomarginal defects.[25]

Considering that the teeth undergoing apical surgery have less predictable prognosis and even a single tooth can be strategic in the whole oral prosthetic rehabilitation, the possibility of accelerating the bone regeneration in periapical surgical defects might be of great interest to the clinician, in order to proceed sooner with the permanent rehabilitation.

The aim of the present one-year follow-up pilot study is to evaluate the radiographic healing and the postoperative discomfort in patients undergoing apical surgery, either by leaving the apical surgical cavity empty or by filling it with the PRF gel. The null hypotheses were that periapical surgical defects filled with the PRF gel require the same healing time of sites treated by conventional surgical techniques and that the patients experienced the same postoperative discomfort with or without PRF application.

Materials and methods

Patient selection

In this study 11 patients underwent endodontic surgery for the treatment of refractory periapical periodontitis. The whole experimentation was conducted in accordance with the declaration of Helsinki of 1983. The patients involved were fully informed about the intent and the design of the study and they were asked to give their approval by signing a written consent.

Patients with severe systemic disorders (i.e. non-controlled diabetes, immunologic diseases, malignant neoplastic processes), thrombocytopenia or insufficient compliance were excluded from the present study. For inclusion of

Table 1 Patients involved in the study.

Group	Subject	Gender	Age	Tooth
Control group	C1	F	45	1.3
	C2	M	72	4.3
	C3	F	37	1.5
	C4	M	60	2.3
PRF test group	T1	F	45	2.5
	T2	M	43	1.5
	T3	F	44	1.1
	T4	F	47	1.2
	T5	M	28	3.6
	T6	M	42	3.7
	T7	F	52	3.2

patients, we selected adult individuals presenting a tooth with persisting periapical radiolucency, the presence of fistula and symptoms after orthograde retreatment and a high risk of jeopardize the root integrity by orthograde approach. Each patient was randomly assigned to the control group ($n = 4$) or PRF test group ($n = 7$) by simple computerized randomization procedures. Detailed information about the patients involved in the study are reported in Table 1.

Surgical procedure

A single surgeon performed all surgical interventions under operating microscope magnification. Lidocaine with epinephrine 1:50,000 was employed as local anaesthetic. Twenty minutes were waited for the vasoactive agent to constrict the local blood vessels in order to achieve optimal haemostasis.[3] Surgical access to the apical area of the involved tooth was obtained via a full-thickness muco-gingival flap with vertical releasing incisions. The bone around the root apex was removed with a round bur mounted on a low-speed handpiece under constant water irrigation. All granulomatous tissue was removed by manual curettage. Ferric sulphate (Astringedent, Ultradent, South Jordan, UT, USA) was used as haemostatic agent. The root was sectioned 3 mm from the anatomical apex. The root-end cavity was performed by using ultrasonic tips (KiS, Spartan Obtura, Fenton, MI, USA), dried with sterile paper points (Inline, BM Dentale, Turin, Italy) and filled with SuperEba (Regular setting powder, Bosworth, Skokie, IL, USA). After the cement setting, the apical surface of the resected root was dyed with methylene blu and a surgical mirror was employed to verify the absence of visible marginal defects. At the end of root-end filling procedure the surgical site was abundantly flushed with saline to remove blood clots and ferric sulphate residuals. In the four patients of the control group, the bone defect was not filled and the flap was sutured with 5×0 and 6×0 monofilament polypropylene. In the other 7 cases (PRF test group), PRF gel was prepared as described below and applied in the bone defect before repositioning and suturing the flap (Fig. 1). Antibiotics were prescribed during the 6 days postoperatively (1 g amoxicillin every 12 h). The choice to assume analgesics was left to the patient. Sutures were removed within 48—72 h from surgery.

PRF preparation

The PRF gel was obtained by following the protocol by Choukroun et al.[26] This consisted of collecting a small amount

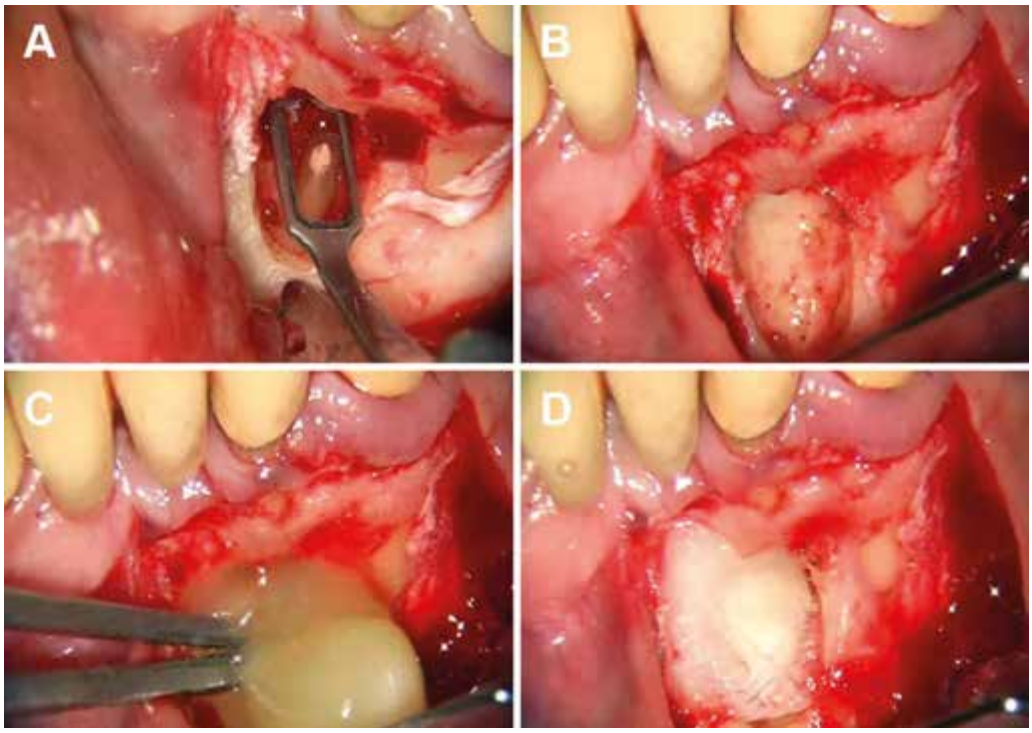

Figure 1 Phases of the PRF gel application: (A) check of the proper seal of the retrograde filling with a surgical mirror; (B) appearance of the periapical bony defect; (C) positioning of the PRF gel in the bony defect with forceps; (D) adaptation of the gel to the defect after compression with a gauze.

of the patient's blood (10—40 mL in the case of our study) at the needed moment of the surgical operation into dried monovettes without anticoagulant agent (Vacuette, Greiner Bio-One, Kremsmünster, Austria). The collected blood was immediately centrifuged for 10 min at 2,500 rpm. The produced clot was extracted from the container by using thin sterile forceps and entirely employed, without depriving it of the red thrombus.

Radiographic centering and examination

Customized filmholders and digital X-ray system (Vistascan Dental Perio, Dürr Dental AG, Bietigheim, Germany) were used throughout the study with a paralleling technique;[27] the X-ray device (2200 Intraoral X-Ray System, Kodak Dental Systems, Rochester, NY, USA) was set at 70 kVp, 10 mA, and 0.20 s exposure time. Radiographs were taken before and after surgery, and at each follow-up visit; recalls were planned at the 1st, 2nd, 3rd, 4th, 5th, 6th and 12th month after surgery.

Two endodontists with 16 and 20 years of clinical experience extraneous to involved patients and study design were calibrated.[28,29] All radiographs were blindly examined twice at interval of at least 30 days. Inter- and intraobserver reproducibility was assessed by means of Kappa statistics.[30] Each follow-up radiograph was assigned to the appropriate category of the classification introduced by Molven et al.:[28,29] complete, incomplete, uncertain or unsatisfactory healing (failure). Independently of the radiological periapical condition, the presence of postoperative clinical complications (e.g. sinus tract, apicomarginal communication, infection with tenderness to palpation or percussion) reported at any time of the control visits was considered as failure.

Pain and swelling assessment

The model for subjective data collection described by Pennarocha et al.[31] was adopted. Each patient was asked to fill out a questionnaire in which pain and swelling information were recorded after 2, 6, and 12 h from the intervention, and each day during the first 7 postoperative days. Pain was rated as follows:

- 0, absence of pain;
- 1 (mild), recognizable but not discomforting pain that did not require the assumption of analgesics;
- 2 (moderate), discomforting but bearable pain that is effectively relieved by analgesics, if assumed;
- 3 (severe), pain that is difficult to bear.

The following scale was formulated to score the postoperative swelling:

- 0, absence of swelling;
- 1, minor intraoral oedema localized to the surgical site;
- 2, moderate extraoral swelling in the surgical zone;
- 3, severe extraoral swelling beyond the treated area.

Statistical analysis

The Statistical Package for Social Sciences v. 15 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics of the considered variables were performed. The significance of the differences between the groups in terms of periapical healing, pain and swelling scores was assessed by means of a Mann-Whitney test. Since the present pilot study was conducted on a restricted number of patients, a p value less than 0.01 was regarded as statistically significant.

Results

The distribution of the periapical healing scores is showed in Fig. 2. After the 1st radiographical recall, only one patient of the PRF test group was classified as healed by the blinded examiners and no significant differences were found between the two groups. At the recalls after 2 and 3 months from the surgical intervention, the PRF test group exhibited significantly better periapical healing scores than the control group. From that moment on, the periapical healing scores of the control and test group were similar and no significant difference was pointed out by the statistical analysis.

The graphs in Figs. 3 and 4 represent the changes in postoperative pain and swelling during the time of observation hours and days after the surgical intervention. By considering both investigated parameters, average to low scores were registered in the two groups with a trend of lower scores associated with the application of PRF. More specifically, the patients of the PRF test group felt less intense pain than the control group during the first hours and days postoperatively,

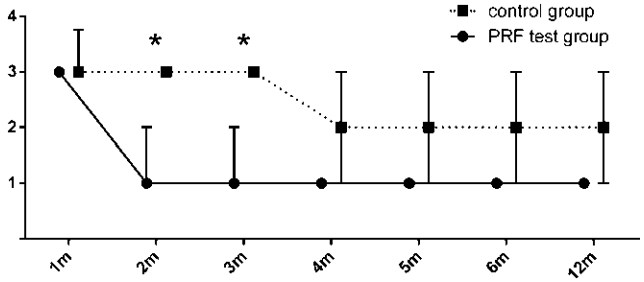

Figure 2 Median values and interquartile ranges of periapical healing scores after months (m) from the surgical intervention: 1, complete healing; 2, incomplete healing; 3, uncertain healing; 4, unsatisfactory healing. The asterisks mark statistically significant differences between control and PRF experimental groups at the specific time point ($p < 0.01$).

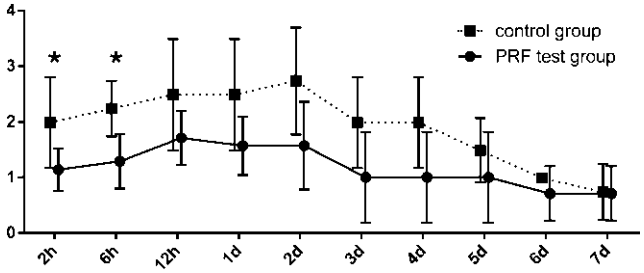

Figure 3 Mean values and standard deviations of pain scores after hours (h) and days (d) from the surgical intervention: 0, absence of pain; 1, mild pain; 2, moderate pain; 3, severe pain. The asterisks mark statistically significant differences between control and PRF experimental groups at the specific time point ($p < 0.01$).

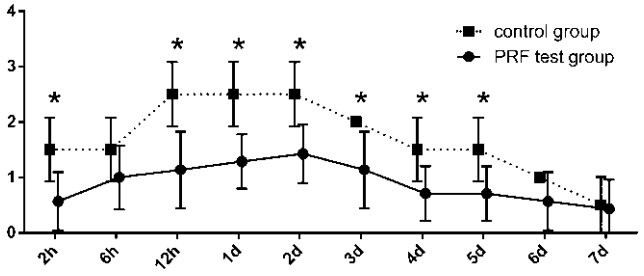

Figure 4 Mean values and standard deviations of swelling scores after hours (h) and days (d) from the surgical intervention: 0, absence of swelling; 1, minor intraoral swelling; 2, moderate extraoral swelling; 3, severe extraoral swelling. The asterisks mark statistically significant differences between control and PRF experimental groups at the specific time point ($p < 0.01$).

with significantly lower scores after 2 and 6 h ($p < 0.01$). As to the swelling assessment, the maximum scores in both groups were reached in the time period between the 12 h and the second postoperative day. Starting from the third postoperative day, the swelling slowly decreased in both groups. The score in the PRF test group never exceeded the moderate intraoral swelling.

Discussion

The present study evaluated the effects of the application of PRF in endodontic surgery. Similar periapical healing scores were assigned to the two groups at the first radiographic control; true to form, one month was not enough to observe mineralization changes with intraoral radiography, even in the PRF test group. On the contrary, radiographic healing in PRF test group appeared to be significantly improved after two and three months from the surgical intervention. If confirmed by studies involving a larger number of patients, such an advantage is likely to arouse the interest of the clinician, because the use of the PRF gel seems to accelerate the healing process, which was detectable earlier. In many clinical and operative situations the possibility to shorten the follow-up period to only few months before proceeding with the permanent rehabilitation would be a substantial asset. Moreover, a general trend of reduced postoperative pain and local swelling was noticed and must be considered in the management of the patient's overall comfort. In the first hours postoperatively the majority of the patients belonging to the test group experienced only mild pain (i.e. bearable without assuming drugs); this means that their use of analgesics could be limited and has both clinical and economic advantages. A clinical trial reported a beneficial effect of the use of plasma rich in growth factors (PDGF) during endodontic surgery in affecting postoperative symptoms and patient's quality of life after surgery.[32] The authors described in detail the procedure to obtain and apply this plasma concentrate to the surgical site, which required several steps to be performed, namely separating the plasmatic component in two fractions, storing them, adding $CaCl_2$ to enable clot formation and finally apply liquid and a clot of PRGF in three steps.[32] We chose to use PRF over PRGF because the former has simpler procedures for both preparation and positioning that allow to spare operative time potentially without compromising the clinical effectiveness.

One of most common indications to apical surgery is the presence of an obstruction that does not allow the access to the entire endodontic space and cannot be overcome. The orthograde techniques for overstepping the obstruction depend on the availability of specific instruments and above all on the operator's dexterity, so that they are hardly standardizeable.[4] Thus, it can be concluded that there are no absolute indications to apical surgery,[33] as they depend on a host of factors. The benefits that PRF could provide in terms of accelerated radiographic healing and limited postoperative soreness might influence the therapeutic choice.

Preferring PRF over PRP in endodontic surgery depends on several factors. Since in most cases the surgical bone defect is likely to be small, PRF, differently from PRP, would be the first choice because it requires the collection of very few milliliters of blood. PRP is obtained from the patient's own blood to whom citrate dextrose solution A is added prior to centrifuging.[34,35] PRF was specifically created for oral and maxillofacial surgery.[26] The preparation of PRF requires neither anticoagulant in the container nor addition of gelling agent (i.e. bovine thrombin).[36] The absence of anticoagulant implies the need of fast transfer and immediate centrifugation of collected blood because fibrin polymerization is not inhibited. During the first centrifugation phase, fibrinogen concentrates in the upper part of the tube; thereafter, circulating thrombin causes the slow transformation of fibrinogen into fibrin and the clot forms in the middle of the tube.[36] Red corpuscles sediment at tube's bottom, whilst acellular plasma supernatant collects at its top. The slow gelling process distinguishes PRF by PRP and other plasma derivates as it modifies the mechanical and biological characteristics of the fibrin matrix.[37] In fact, physiological thrombin concentration determines the organization of the fibrin network in a biochemical architecture characterized by trimolecular or equilateral junctions between monomers.[36] This three-dimensional structure allow the establishment of a flexible, elastic and resistant PRF gel, in which cytokines are retained and cellular migration is supported by the fibrin network.[38,39] Platelets are mainly entrapped in the clot at the interface between the fibrin clot and its lower portion (the red thrombus); thus, this portion of the plasma derivate gains in clinical relevance because of the substances it contains.[38] PRF seems also capable of enmeshing glycosaminoglycans,[38] whose affinity for circulating platelet cytokines can enhance the cell migration and the healing process.[40] Cytokines are soluble molecules that play a relevant role in healing and regeneration mechanisms in injured tissues;[38,41] their capability of regulate inflammation and healing phenomena consists of a multitude of molecular interactions that has not been completely understood and described.[39] The biologic activity and clinical effectivess of the PRF gel benefits from a partially known cytokines action. Our preliminary results highlight a trend of lower postoperative discomfort and accelerated bone healing in the patients who received the PRF gel; these findings can be indicative of a attenuated inflammatory response and enhanced healing of the surgical site. The action of healing cytokines the PRF gel contains, consists of interrupting the inflammation process or promoting angiogenesis. The effects of interleukin 4 (IL-4) profoundly depend on the cytokines environment.[42] When

inflammatory processes are present, IL-4 acts as regulator by inhibiting the IL-1β-mediated signal.[43] The most powerful agent for angiogenesis promotion is the vascular endothelial growth factor (VEGF), which can control growth, migration and differentiation of epithelial cells.[44] PRF has been defined an "immune organizing node" owing to its content in cytokines with both pro- (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-4) action, which was found to be superior than in plasma concentrates.[39]

Although cytokines and cells enmeshed in PRF fibrin network influence tissue healing, the molecular fibrin structure seems to be the crucial characteristic of PRF.[15] It is noteworthy that fibrin employed in surgery as a single agent cannot lead to sufficient bone regeneration.[45] The complex fibrin matrix of PRF can induce angiogenesis, because endothelial cells can migrate and adhere to its articulate structure in which they differentiate and proliferate.[46] Moreover, one of the main angiogenesis soluble factors, the platelet-derived growth factor (PDGF), binds to fibrin with high affinity.[47,48]

Since PRF has been introduced in recent years, only a small number of clinical studies on its efficacy have been produced; nevertheless, hopeful results have been obtained in different fields of oral surgery.[21,49–51] Some clinical case reports or series have been produced on the PRF application to endodontic surgery, with the authors generally describing reduced morbidity and discomfort for the patient and accelerated healing.[22–24] Nevertheless, no effort is made in these studies to standardize the surgical techniques or the preoperative conditions;[23] moreover, some authors make use of bone substitutes – e.g. hydroxyapatite or β-tricalcium phosphate (TCP)[22,24] – which are likely to affect the reliability of the radiographic assessment. On similar basic principles, also a case report of a single periapical lesion treated with PRP and allogenic graft (TCP) has been published.[52] The authors speculated that PRP could accelerate TCP resorption and reported a subtotal replacement of the grafting material with newly-formed bone 12 months after surgery. Since a 3-year follow up study on 146 teeth that underwent standard periapical surgery reported that 66% of treated teeth could be considered healed after 12 months,[7] the use of PRP and TCP as grafting material appears questionable at the moment. Recently, a randomized controlled trial on the surgical treatment of apicomarginal defects with PRF has been published.[25] However, endoperiodontal defects are peculiar lesions, whose treatment is known to be particularly arduous. The authors did not find significant benefit from the use of PRF. Differently, the aim of the present study was to assess the effect of PRF in endodontic lesions without periodontal communication, so it is probable that the gel can express its beneficial effect in the absence of bacterial interference from the marginal periodontum and other non-controlled factors.

Similarly to the case of periodontal regenerative therapy, bone grafting materials have been frequently employed in endodontic surgery to promote bone regeneration and their ability to induce new bone formation has been described well.[53] Notwithstanding, the risk of ankylosis after the use of a grafting material in endodontic surgery has still to be assessed because grafting materials might interfere with the regeneration of the periodontal ligament. There are no specific indications to bone grafting in periapical surgery on account of their controversial ability to provide favourable healing and because of a lack of controlled clinical trials.[53] PRF is an autologous material that surmounts problems related to graft rejection, which might occur when the source of the grafting material is allograft, alloplast or xenograft.

Conclusions

The application of PRF gel in apical surgery showed promising result in stimulating bone formation after 2 and 3 months around periapical surgical defects and in reducing postoperative discomfort. Further clinical studies are needed to confirm the findings of this pilot study.

Conflict of interest

The authors have no conflict of interest to declare.

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