Journal of the Formosan Medical Association (2014) 113, 470—476

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.jfma-online.com

CASE REPORT

Management of radicular cysts using platelet-rich fibrin and bioactive glass: A report of two cases

Jiing-Huei Zhao [a], Chung-Hung Tsai [b], Yu-Chao Chang [a,c,*]

[a] School of Dentistry, Chung Shan Medical University, Taichung, Taiwan
[b] Department of Oral Pathology, Chung Shan Medical University Hospital, Taichung, Taiwan
[c] Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan

Received 16 June 2011; received in revised form 5 September 2011; accepted 8 September 2011

KEYWORDS
bioactive glass;
platelet-rich fibrin;
radicular cyst;
tissue regeneration

Platelet-rich fibrin (PRF) created by Choukroun's protocol concentrates most platelets and leukocytes from a blood harvest into a single autologous fibrin biomaterial. However, no current data is available concerning the use of PRF for the treatment of periapical lesions. Two cases of radicular cysts were reported using an interdisciplinary approach, including regular endodontic therapy followed by surgical management with PRF and bioactive glass. Two cases of radicular cysts presented as an incidental radiographic finding, appearing as an apical radiolucency with well-circumscribed sclerotic borders. After regular endodontic retreatment, cystic lining/granulation tissues were enucleated and the periradicular bony defect was grafted using PRF and bioactive glass. Then, PRF was applied to serve as a membrane over the grafted defects. Recall periapical radiographs of Case 1 and cone beam computer tomography of Case 2 showed satisfactory healing of the periapical pathosis. In Case 2, the bony defect appeared completely healed at 4 months surgical reentry and the new bone was clinically very dense and mature. The results of these case reports show that the combination of PRF and bioactive glass is an effective modality of regenerative treatment for radicular cysts.

Introduction

Although regular endodontic therapy can be used with predictability to arrest mild to moderate defects, it might be inadequate for the wide circumferential apical defects caused by endodontic infection. Although traditional

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. School of Dentistry, Chung Shan Medical University, 110, Section 1, Chien-Kuo North Road, Taichung, Taiwan.
E-mail address: cyc@csmu.edu.tw (Y.-C. Chang).

0929-6646/$ - see front matter
doi:10.1016/j.jfma.2011.09.027

surgical procedures provide better access in these situations, there is still a disadvantage to both techniques, in that tissue repair is the probable outcome. Endodontic regenerative procedures frequently include the use of barrier membranes and bone grafting materials to encourage the growth of key surrounding tissues.[1]

Platelet-rich fibrin (PRF) described by Choukroun et al.[2] is a second-generation platelet concentrate that allows one to obtain fibrin membranes enriched with platelets and growth factors, after starting from an anticoagulant-free blood harvest. The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and leucocytes of the blood harvest[3,4] and creates a complex architecture as a healing matrix, including mechanical properties no other platelet concentrate offers. It is an autologous biomaterial, not an improved fibrin glue. Recently, we reported that PRF could stimulate cell proliferation of osteoblasts,[5,6] gingival fibroblasts,[5] pulp cells,[7] and periodontal ligament cells,[5] but suppress oral epithelial cell growth.[5] These cell-type-specific actions of PRF may be beneficial for tissue regeneration. Some clinical applications in socket preservation,[8,9] sinus augmentation,[10,11] and periodontal regeneration surgery have been described previously.[12,13] However, no data are available on the use of PRF in the treatment of periapical lesions.

Bone grafting materials have been used to maintain the space for cell repopulation and to act as osteoinductive or osteoconductive materials for the formation of host bone.[14,15] Bioactive glass is used widely in other clinical situations, such as periodontal lesions. Bioactive glass has been demonstrated to repair periodontal defects and to produce bone fill in the defect. Upon implantation, the material immediately interacts with the patient's body fluid and elicits a series of reactions that include leaching, dissolution, and precipitation, to form a silica- and calcium-rich surface gel that traps cellular and noncellular materials within the gel matrix. Within the matrix, hydroxycarbonate-apatite nucleates crystallize and interpose with mucopolysaccharides, glycoproteins, collagen, and osteo-cellular materials. With time, the "living" matrix is transformed, remodeled, and replaced by newly formed osseous tissue.[16,17]

The aim of this case report is to describe two cases of radicular cysts in the maxillary anterior region that were individually managed, depending on the underlying etiology, by using an interdisciplinary approach including nonsurgical restorative and endodontic therapy followed by surgical management with PRF and bioactive glass.

Case 1

A 28-year-old female patient attended the Oral Medicine Center of Chung Shan Medical University Hospital with pain in the maxillary anterior region. There was no previous history of pain or discomfort. The patient's medical status

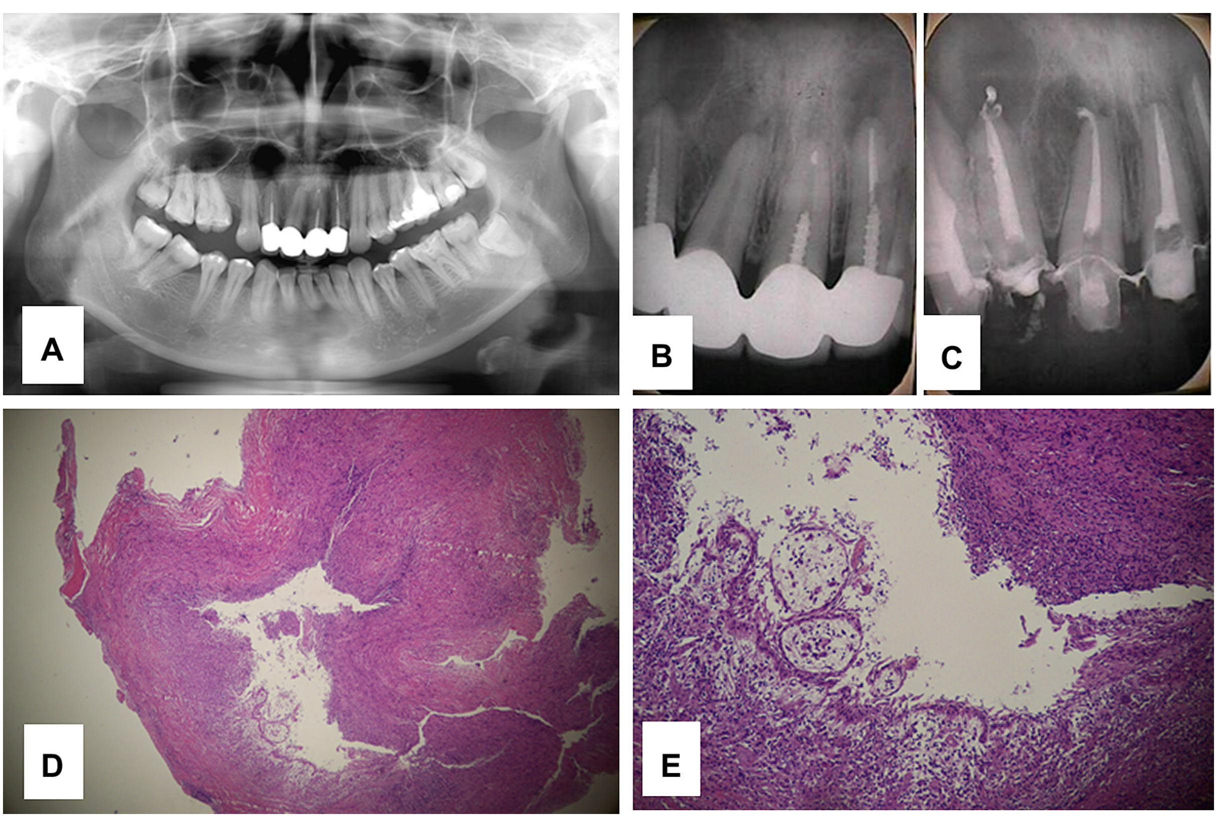

Figure 1 (A) Panoramic radiographic examination shows incomplete root canal fillings of teeth #7, #9, #10 and apical radiolucency over #6, #7, #8, and #10; (B) periapical radiograph examination shows apical radiolucency over #7, #8, and #10; (C) periapical radiograph of teeth #7, #8, #9, and #10 after root canal retreatment; (D) pathological examination shows a radicular cyst with desquamated lining epithelium; (E) the cystic wall with dense acute and chronic inflammatory cell infiltration.

was noncontributory, and she mentioned that she had no trauma to the related area. An existing crown bridge restoration was noted from the right maxillary lateral incisor to the left maxillary lateral incisor. The teeth responded positively to percussion. The radiographic examination revealed incomplete root canal fillings of the right maxillary lateral incisor, the left maxillary central incisor, and the left maxillary lateral incisor. (Fig. 1A). In addition, apical radiolucency with a well-circumscribed sclerotic border was found over the right maxillary canine, the right maxillary lateral incisor, the right maxillary central incisor, and the left maxillary lateral incisor (Fig. 1A and 1B).

After the screw posts were removed from the right maxillary lateral incisor, the left maxillary central incisor, and the left maxillary lateral incisor, nonsurgical endodontic retreatment was performed by removing the existing gutta-percha, determining the working length after tooth isolation by rubber dam, copious irrigation with 2.5% sodium hypochlorite, and interappointment calcium hydroxide root canal medication. The electronic pulp sensitivity test was negative for the right maxillary canine, which had also received root canal treatment. Four weeks later, the root canals were obturated with gutta-percha and sealer, with radiographic confirmation of a satisfactory obturation (Fig. 1C).

The second step of the treatment was to manage the periapical lesion surgically. Using intrasulcular incision and vertical release, a full-thickness flap was reflected and thin cortical bone was removed to create the bony window. The cystic lining/granulation tissue was totally removed (which was sent for biopsy), followed by curettage of each defect. Microscopic examination showed the cystic cavity with partly desquamated lining epithelium (Fig. 1D). The cystic wall was thickened, with dense acute and chronic inflammatory cell infiltration including neutrophils, eosinophils, lymphocytes, plasma cells, fibroblasts, and foamy cells (Fig. 1E). An apicoectomy was performed to remove 2—3 mm of root end using a high-speed fissure bur. After retrograde cavity preparation, amalgam was used for retrograde filling.

Blood samples were taken according to the PRF protocol,[3] with a PC-02 table centrifuge and collection kits provided by Process (Nice, France). Quickly, blood samples were taken from this patient without using an anticoagulant, in 10-ml glass-coated plastic tubes (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and immediately centrifuged at 3000 rpm for 12 minutes. As shown in Fig. 2A, a fibrin clot formed in the middle part of the tube, while the upper part contained acellular plasma, and the bottom part contained red corpuscles. The fibrin clot was separated easily from the lower part of the centrifuged blood. The PRF clot was pressed gently into a membrane with sterile dry gauze. PRF membranes were minced as graft materials and trimmed as membranes (Fig. 2B). Resorbable bioactive glass (Unigraft; Unicare Biomedical, Laguna Hills, CA) mixed with the minced PRF were carried and packed into the defects (Fig. 2C). Then, the PRF membrane was applied over the grafted defects (Fig. 2D). Wound closure was then obtained with 4-0 silk sutures. Analgesics and antibiotics were prescribed, and

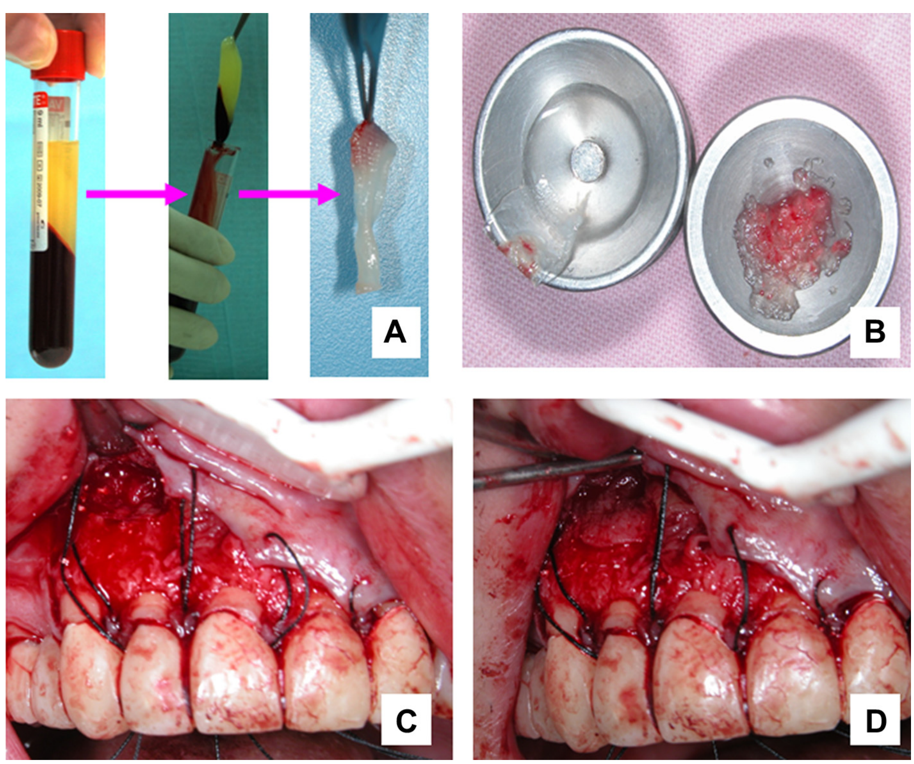

Figure 2 (A) PRF formed in the middle part of the tube. The fibrin clot separated easily from the lower part of the centrifuged blood. The PRF clot was gently pressed between two layers of sterile dry gauze to form a membrane. (B) PRF membrane (left); minced PRF mixed with bioactive glass (right); (C) minced PRF/bioactive glass were packed tightly into the bony defects; (D) the PRF membrane was applied to cover the bony defects.

the patient was advised to use chlorhexidine mouthwash for a week. The sutures were removed after 7 days.

Biopsy results in the present case confirmed the diagnosis of a radicular cyst. Clinical healing was uneventful, with neither infectious episodes nor untoward clinical symptoms. The patient was recalled once in a month postoperatively within 7 months. Follow-up radiographs at 1 month (Fig. 3A and 3B) and 7 months (Fig. 3C and 3D) showed satisfactory bone healing.

Case 2

A 27-year-old female patient presented with a failing crown and bridge in the maxillary anterior region. On clinical examination, there was grade "I" mobility with the maxillary right incisor central, the lateral incisor, and the canine, and swelling in the buccal aspect of the same area. Panoramic radiographic examination revealed incomplete root canal fillings of the right maxillary first premolar to the left maxillary central incisor and a well-defined radiolucency with well-circumscribed sclerotic borders extending from the right maxillary canine to the left maxillary central incisor periapical regions (Fig. 4A).

The sagittal view of cone beam computer tomography (CBCT) revealed that the radiolucency area was about 10 mm in the buccal-palatal direction (Fig. 4B). The extent of periapical radiolucency was 16 x 17 mm, measured by the panoramic view of the CBCT (Fig. 4B).

The nonsurgical endodontic retreatment and apical surgery were similar as in Case 1 (Fig. 5A,5B and 5C). The pathologic examination also was documented as a radicular cyst. The patient was recalled once a month postoperatively. Four months after surgery, surgical reentry was

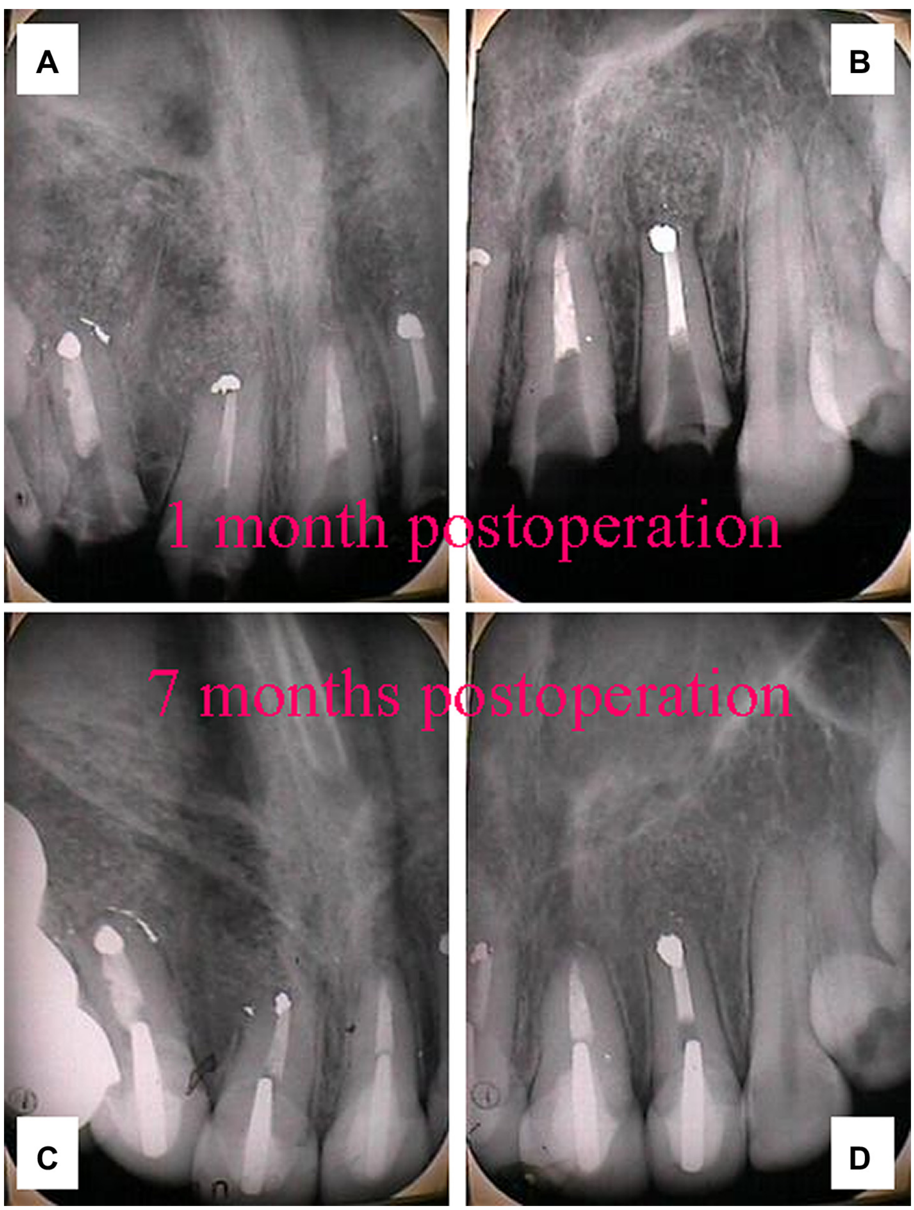

Figure 3 Follow-up radiographs at 1 month showed satisfactory healing (A and B); recalls at 7 months' postoperatively showed that osseous healing was almost complete in the cystic lesions (C and D).

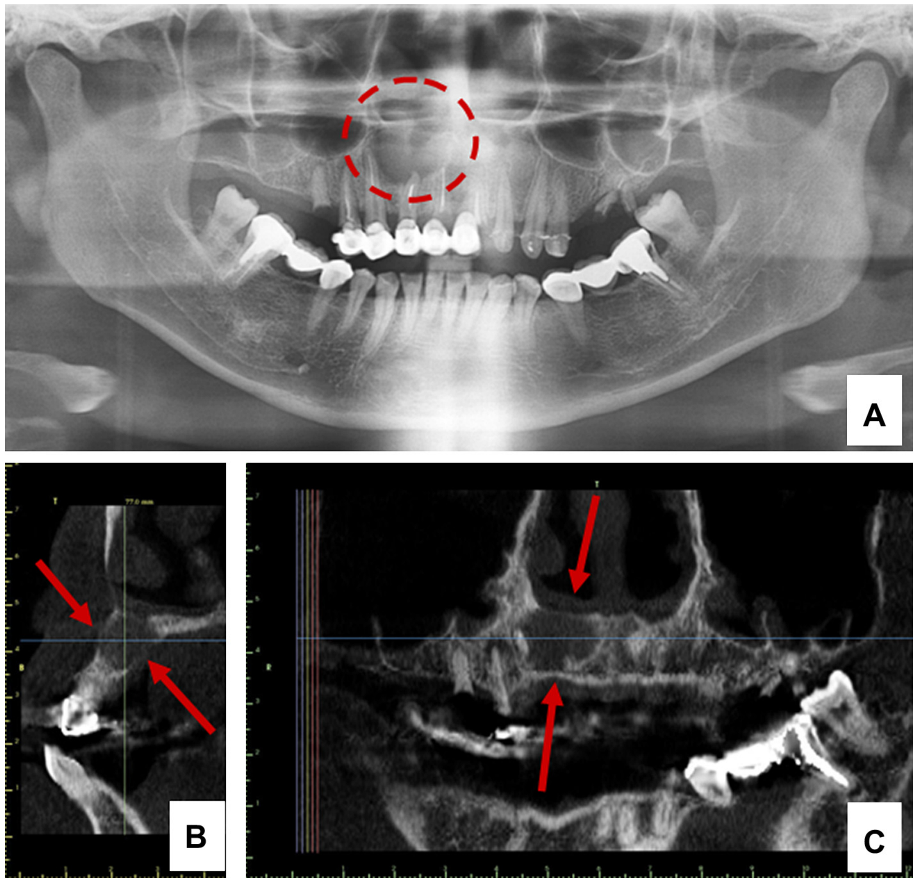

Figure 4 (A) Panoramic radiographic examination revealed incomplete root canal fillings of #5 to #9, and a well-defined radiolucency with well-circumscribed sclerotic borders around the apex of #6, #7, and # 8; (B) CBCT revealed bony radiolucency in the buccal-palatal direction; (C) periapical radiolucency shown by the panoramic view of the CBCT.

reevaluated through an invasive surgical procedure to measure the defect fill. The bony defect was filled completely with tissue of osseous appearance, which on probing exhibited the consistency of bone (Fig. 5D). CBCT examination showed the cystic lesion filled almost totally with new bone tissue (Fig. 6A and 6B).

Discussion

PRF prepared using Choukroun's technique, is prepared naturally, without the addition of thrombin. It is hypothesized that the PRF has a natural fibrin framework that can protect growth factors from proteolysis.[18] It is organized as a dense fibrin scaffold,[19] with a specific slow release of growth factors.[20] PRF can be considered as a natural fibrin-based biomaterial to guide cell migration into the wound. In addition, growth factors are active for a relatively longer period and are effective in stimulating tissue regeneration. This leads to the idea of using PRF as a biomaterial for periapical tissue regeneration.

To the best of our knowledge, this is the first report of the use of PRF and bioactive glass for the treatment of radicular cysts. Recall examinations of the patients who received the two procedures, whether by periapical radiographs or CBCT, demonstrated resolution of the original lesions. Radiographic evaluation is a noninvasive examination for bony defects repair. However, bone fill data derived from surgical reentry are important to substantiate routine postoperative measurement data.

In Case 2, the second surgical procedure involved flap elevation, revealing the presence of hard tissue underneath the gingival tissues. However, the histology of the treated periapical bony defects was the only reliable method to determine the nature of the periapical soft and hard tissue interface.

The reason PRF can improve periapical osseous healing can be explained as follows. This fibrin matrix can guide the healing processes. Recently, we found that PRF can upregulate phosphorylated extracellular signal-regulated protein kinase expression and suppress osteoclastogenesis by promoting the secretion of osteoprotegerin (OPG) in osteoblasts cultures.[6] PRF also was demonstrated to stimulate osteogenic differentiation of human dental pulp cells[7] and periodontal ligament cells[13] by upregulating OPG and alkaline phosphatase expression.

Many growth factors, such as platelet-derived growth factor and transforming growth factor, are released from the PRF.[3,18] In addition, PRF may play an important role in the revascularization of the graft by supporting angiogenesis.

When all of this is considered, PRF can be recognized as an autologous biomaterial. PRF as a membrane and grafting material offers an improved space-making effect on the barrier, which is conducive to cell events leading to

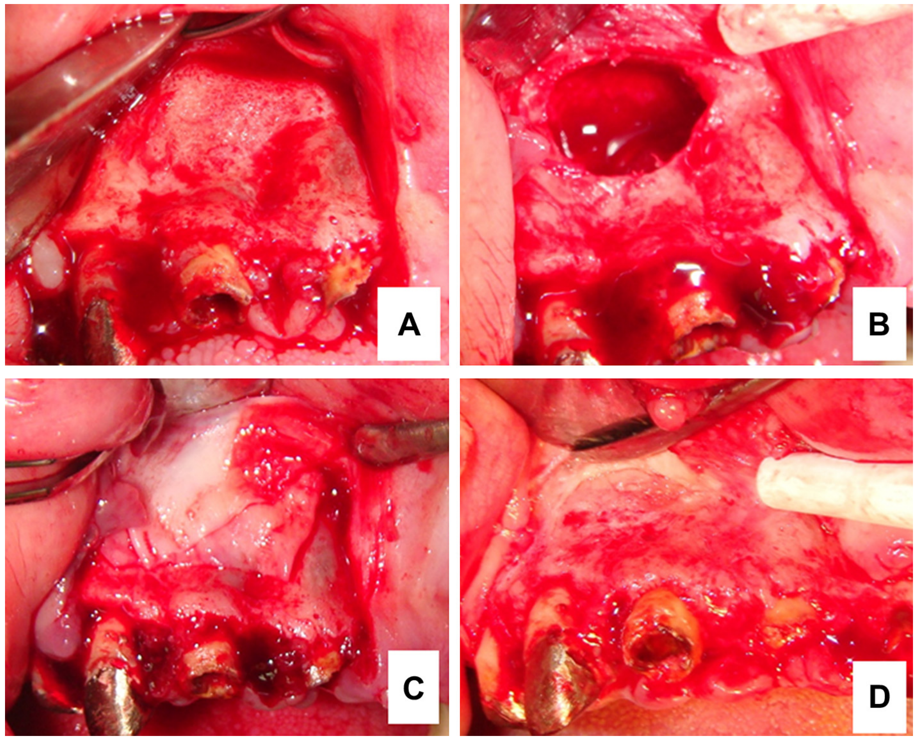

Figure 5 (A) Flap elevation over the apical region of teeth #6, #7, and #8. (B) After removal of the apical cyst-like tissue, the bony defects over the apical region of teeth #6, #7, and #8 were noted. (C) Minced PRF/bioactive glass were tightly packed into bony defects and covered with PRF membranes. (D) Surgical reentry revealed that the bony defect was completely filled with osseous tissue.

periapical tissue regeneration and facilitation of mineralized tissue formation due to osteoconductive and/or osteoinductive properties possibly inherent in PRF. Thus, this preparation could lessen the treatment duration and may assist in the decision making of upcoming implant or prosthetic processes. It also could be developed as an option coupling for conventional surgical removals.

Treating periodontal intrabony defects with bioactive glass bone grafting material has yielded clinically acceptable responses.[21] Bioactive glass also has been found to exhibit an antibacterial effect against subgingival and supragingival oral bacteria, and it may reduce implant-centered infections.[22] No systematic review regarding tissue regeneration in endodontic lesions exists. However, the best results could be obtained by using it in both membranes and bone grafts.[23] It is evident that the design employed in the current study does not allow for determining whether PRF or bioactive glass is responsible for the improved results observed with tissue regeneration. The clinical use of PRF as the sole grafting material in periapical bony defect will need to be studied in further cases.

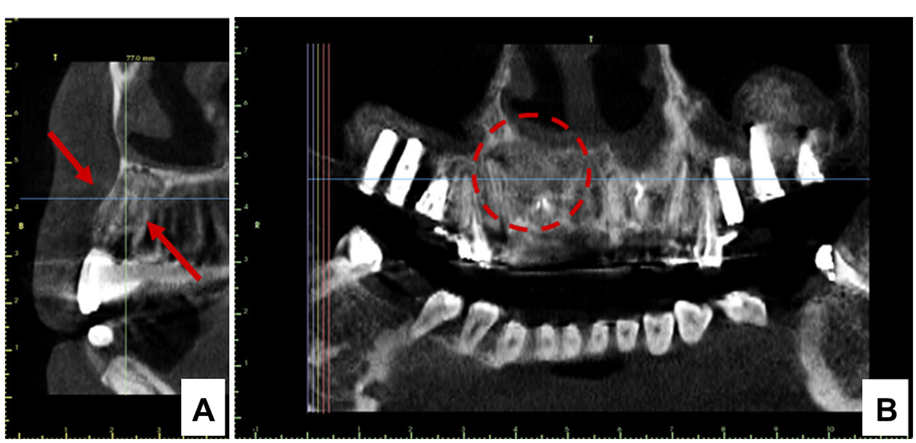

Figure 6 Four months after surgery, CBCT examination showed that the cystic lesion has been almost totally filled with a bone-looking dense tissue in the sagittal view (A) and the panoramic view (B).

PRF via Choukroun's technique is simple and inexpensive, and the systematic use of this biomaterial for endodontic regeneration seems a very promising option. This case report demonstrates that the combination of PRF and bioactive glass did not appear to "prevent" healing of the apical lesion in the two reported cases. In addition, it showed satisfactory healing of the periapical pathosis and it positively influenced the apical osseous tissue. As a short case series, this is an innovative topic, but its clinical value is somewhat debatable at this stage as no definitive conclusions can be drawn from two case reports. In addition to the recruitment of other extensive cyst cases, further controlled clinical trials are necessary to determine whether the addition of PRF alone or in combination significantly enhances bone formation and maturation in cyst lesions.

References

1. Bashutski JD, Wang HL. Periodontal and endodontic regeneration. *J Endod* 2009;**35**:321–8.
2. Choukroun J, Adda F, Schoeffer C, Vervelle A. PRF an opportunity in perio-implantology (in French). *Implantodontie* 2000; **42**:55–62.
3. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:E45–50.
4. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:E51–5.
5. Tsai CH, Shen SY, Zhao JH, Chang YC. Platelet-rich fibrin modulates cell proliferation of human periodontally related cells in vitro. *J Dent Sci* 2009;**4**:130–5.
6. Chang IC, Tsai CH, Chang YC. Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. *J Biomed Mate Res A* 2010;**95**:327–32.
7. Huang FM, Yang SF, Zhao JH, Chang YC. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. *J Endod* 2010;**36**:1628–32.
8. Simon BJ, Gupta P, Tajbakhsh S. Quantitative evaluation of extraction socket healing following the use of autologous platelet-rich fibrin matrix in humans. *Int J Periodontics Restorative Dent* 2011;**31**:284–95.
9. Zhao JH, Tsai CH, Chang YC. Clinical and histologic evaluation of extraction socket healing filled with platelet rich fibrin. *J Dent Sci* 2011;**6**:116–22.
10. Toffler M, Toscano N, Holtzclaw D. Osteotome-mediated sinus floor elevation using only platelet-rich fibrin: an early report on 110 patients. *Implant Dent* 2010;**19**:447–56.
11. Simonpieri A, Choukroun J, Del Corso M, Sammartino G, Dohan Ehrenfest DM. Simultaneous sinus-lift and implantation using microthreaded implants and leukocyte- and platelet-rich fibrin as sole grafting material: a six-year experience. *Implant Dent* 2011;**20**:2–12.
12. Chang YC, Wu KC, Zhao JH. Clinical application of platelet-rich fibrin as the sole grafting material in periodontal intrabony defects. *J Dent Sci* 2011;**6**:181–8.
13. Chang YC, Zhao JH. Effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects. *Aust Dent J* 2011;**56**:365–71.
14. Gouldin AG, Fayad S, Mellonig JT. Evaluation of guided tissue regeneration in interproximal defects: (II)-membrane and bone versus membrane alone. *J Clin Periodontol* 1996;**23**:485–91.
15. Mellonig JT, Nevins M, Sanchez R. Evaluation of a bioabsorbable physical barrier for guided bone regeneration: part II- material and a bone replacement graft. *Int J Periodontic Restorative Dent* 1998;**18**:129–37.
16. Schepers E, Ducheyne P, Barbier L. Long term clinical evaluation of bioactive glass particles of narrow size range. *Bioceramics* 1996;**9**:99–102.
17. Hench LL. Bioactive materials: the potential for tissue regeneration. *J Biomed Mater Res* 1998;**41**:511–8.
18. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): A gold standard to achieve for all surgical platelet concentrates technologies. *Growth Factors* 2009;**27**:63–9.
19. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. *J Periodontol* 2010;**81**:546–55.
20. Dohan Ehrenfest DM, Diss A, Odin G, Doglioli P, Hippolyte MP, Charrier JB. In vitro effects of Choukroun's PRF (platelet-rich fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts in primary cultures. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;**108**:341–52.
21. Mengel R, Schreiber D, Flores-de-Jacoby L. Bioabsorbable membrane and bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis: results of a 5-year clinical and radiological study. *J Periodontol* 2006;**77**:1781–7.
22. Allan I, Newman H, Wilson M. Anti-bacteria effects of particulate bioglass against sub-and supra-gingival bacteria. *Biomaterials* 2001;**22**:1683–7.
23. Tobon SI, Arismendi JA, Marin ML, Mesa AL, Valencia JA. Comparison between a conventional technique and two bone regeneration techniques in periradicular surgery. *Int Endod J* 2002;**35**:635–41.