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Platelet Rich Fibrin and Nanocrystalline Hydroxyapatite with Collagen Combination in Treatment of Periapical Lesion: A Novel Clinical Approach

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Authors' contributions

This work was carried out in collaboration between all authors. Author HR designed the case study, wrote the protocol, and wrote the first draft of the manuscript. Author AA managed the follow up and final outcome of the study. Authors AC and RB managed the literature searches. All authors read and approved the final manuscript.

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Case Study

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ABSTRACT

Aims: To use platelet rich fibrin (PRF), which is an autologous platelet concentrate, along with nanocrystalline hydroxyapatite with collagen for treatment of periapical lesion and negotiation of calcified canal of adjacent tooth.
Case Presentation: A 19-year-old female reported to the Department of Conservative Dentistry and Endodontics with chief complaint of pain in maxillary right central incisor. Past dental history revealed trauma which she sustained 10 yrs back in the same region. On intraoral examination, there was a draining sinus, in relation to the apex of 11. Also discolouration and crown fracture was

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found in relation to 11. Periapical radiograph revealed a large diffused periapical radiolucency in relation to 11 and 12, open apex of 11 and calcified root canal in relation to 12.
Technique Used in the Study: A periapical surgery under local anaesthesia was planned in the region of maxillary right anterior region in relation to tooth no 11 and 12. Before surgery calcified canal of tooth no 12 was negotiated. PRF and nanocrystalline hydroxyapatite with collagen combination were placed in bony cavity. Follow up of the case was done for period of 6 months.
Discussion: In present case combination of nanocrystalline hydroxyapatite with collagen and PRF is used, as it helps in faster bone regeneration. Graft material is osteoconductive and collagen network provides a better scaffold for clot formation and bone regeneration.
Conclusion: The combination of PRF and nanocrystalline hydroxyapatite with collagen has been demonstrated to be an effective approach to induce faster periapical healing in present case with large periapical lesion.

Keywords: PRF; nanocrystalline hydroxyapatite; pulp canal calcifications; apical periodontitis.

1. INTRODUCTION

Apical periodontitis or periapical lesion is a sequel to endodontic infection and manifests itself as the host defense response to microbial challenge emanating from the root canal system. It is viewed as a dynamic encounter between microbial factors and host defenses at the interface between infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard tissues, destruction of other periapical tissues, and eventual formation of various histopathological categories of apical periodontitis, commonly referred to as periapical lesions [1].

They are generally diagnosed either during routine dental radiographic examination or following acute pain and/or swelling in relation to the affected tooth [2]. Most periapical lesions (>90%) can be classified as dental granulomas, abscesses, or radicular cysts [3,4]. The incidence of cysts within periapical lesions varies between 6 and 55% [5]. The occurrence of periapical granulomas ranges between 9.3 and 87.1%, and of abscesses between 28.7 and 70.07% [6].

Treatment of teeth with pulp canal calcification presents a dilemma. A number of authors have proposed that for endodontic technical reasons and for prevention of tooth discolouration, these teeth should be root filled once the calcification process is detected. Endodontic procedures are certainly easier if undertaken at this stage. However, most of the recent literature indicates that endodontic treatment is unnecessary unless the tooth is symptomatic or there is radiographic evidence of pulp necrosis and infection [7]– i.e., a periapical radiolucency.

Bone graft and bone regenerative materials are being used in periapical surgery with varying degrees of success. However, the key to tissue regeneration is to stimulate a coordinated cascade of healing events that can result in integrated tissue formation. This is possible only with the use of growth factors, extracellular matrix and bone morphogenetic proteins instead of routinely used synthetic bone grafts, as the latter induce bone regeneration by osteoconduction, while the former stimulate regeneration by osteoinduction [8].

Platelet rich plasma (PRP), first generation of autologous platelet concentrate, has been used for the purpose of tissue regeneration [9,10]. Although its use has shown clinical success its complex preparation protocol and moderate benefits limit its usage in regenerative surgeries [11,12].

Platelet rich fibrin (PRF), introduced by Choukroun et al. [12] in the year 2001, is a second-generation platelet concentrate enriched with platelets and growth factors which promote periapical tissue regeneration and healing. Unlike PRP, it is obtained from an anticoagulant and thrombin free blood harvest making it free from the risk of disease transmission.

PRP has been successfully used with bone grafts like β-Tricalcium Phosphate for bone regeneration in the treatment of periodontal defects [13,14].

In the present case an innovative idea of combining PRF with nanocrystalline hydroxyapatite with collagen was used to treat periapical lesion. Indeed, separate studies have shown clinical success in bone formation with the use of both these materials used separately. This case report presents an attempt to evaluate the healing kinetics of the combination of PRF and nanocrystalline hydroxyapatite with collagen as

opposed to using these materials alone, along with that calcified canal of adjacent tooth was also negotiated. Follow up of the case was done both clinically and radiographically for a period of 6 months.

2. CASE PRESENTATION

A 19-year-old female reported to the Department of Conservative Dentistry and Endodontics with chief complaint of pain in maxillary right central incisor. Past dental history revealed trauma which she sustained 10 yrs back in the same region. On intraoral examination, there was a draining sinus, in relation to the apex of 11. Also discolouration and crown fracture was found in relation to 11. On Electric pulp testing, tooth number 12 was also found nonvital. Intra-oral periapical radiograph (IOPA) revealed a large diffused periapical radiolucency in relation to 11 and 12 measuring 1.4cm in diameter, open apex of 11 and calcified root canal in relation to 12 (Fig. 1). It was decided to perform periradicular surgery as this case fit the indication for the same.

The treatment plan was explained to the patient in a simplified manner and informed consent was obtained. In order to achieve optimal healing and regeneration of bone, it was planned to use PRF in combination with bone graft. An ethical clearance was obtained from the institutional ethical committee. The surgical protocol included a routine medical history and blood investigations. Intraoral and extraoral antisepsis was performed using 0.2% chlorhexidine digluconate rinse and povidone iodine solution, respectively. Following administration of local anaesthesia, sulcular incision followed by two vertical relieving incisions were given and a thick mucoperiosteal flap was reflected (Fig. 2a). Meticulous defect debridement was done; 11 and 12 were then obturated using lateral and vertical condensation technique. Apically 3mm retrograde plug of MTA (Mineral Tri-oxide Aggregate) was placed in relation to 11.

PRF was prepared in accordance with the protocol developed by Freymiller and Aghaloo [15]. Intravenous blood (by venipuncturing of the antecubital vein) was collected in a 10mL sterile tube without anticoagulant and immediately centrifuged at 3,000rpm for 10 minutes (Fig. 2c). Blood centrifugation allowed the formation of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (platelet-poor plasma) at the top (Fig. 2b). PRF was easily separated from red corpuscles base (preserving a small RBC layer) using sterile tweezers just after removal of PPP (platelet-poor plasma) and then transferred into a sterile dappen dish.

PRF was placed carefully above the nanocrystalline hydroxyapatite with collagen bone graft (SYBO GRAF^{TM}-C, Eucare Pharmaceuticals Ltd., Chennai, India) and augmented into the intrabony defect upto the surrounding bone level (Fig. 2d,e,f). The mucoperiosteal flap was repositioned and simple interrupted sutures were given using 3–0 nonabsorbable black silk suture. Systemic antibiotics (amoxicillin 500mg, 3 times a day for 5 days) and analgesics (ibuprofen 400mg, 3 times a day for 3 days) were prescribed. The patient was instructed to rinse 0.2% chlorhexidine digluconate for 7 days. The sutures were removed after 7 days. The patient was kept under observations at regular intervals of 1week, 1, 3 and 6months. During recall visits surgical site was evaluated clinically as well as radiographically for proper healing of periapical defect.

In present case, there was an increase in radio-opacity immediately, due to the compaction of the bone graft into the defect. In the first month there was reduction in volume of the material due to initiation of resorption of the bone graft. In the third month there was evidence of bone regeneration, but not complete, due to partial resorption of the graft. In the sixth month bone regeneration was complete because of the combined effect of graft and PRF.

In this case the graft did not produce any adverse reactions. Wound healing and primary closure of the surgical site were good. Radiographic evaluation revealed favorable outcomes regarding bone regeneration and repair of periapical tissues.

The clinical follow-up at 6 months showed the patient functioning well with no reportable clinical symptoms and an absence of any sinus tract formation. The radiographic follow-up showed >90% healing of the periapical radiolucency and bone regeneration with evidence of a trabecular pattern at the end of 6 months (Fig. 3).

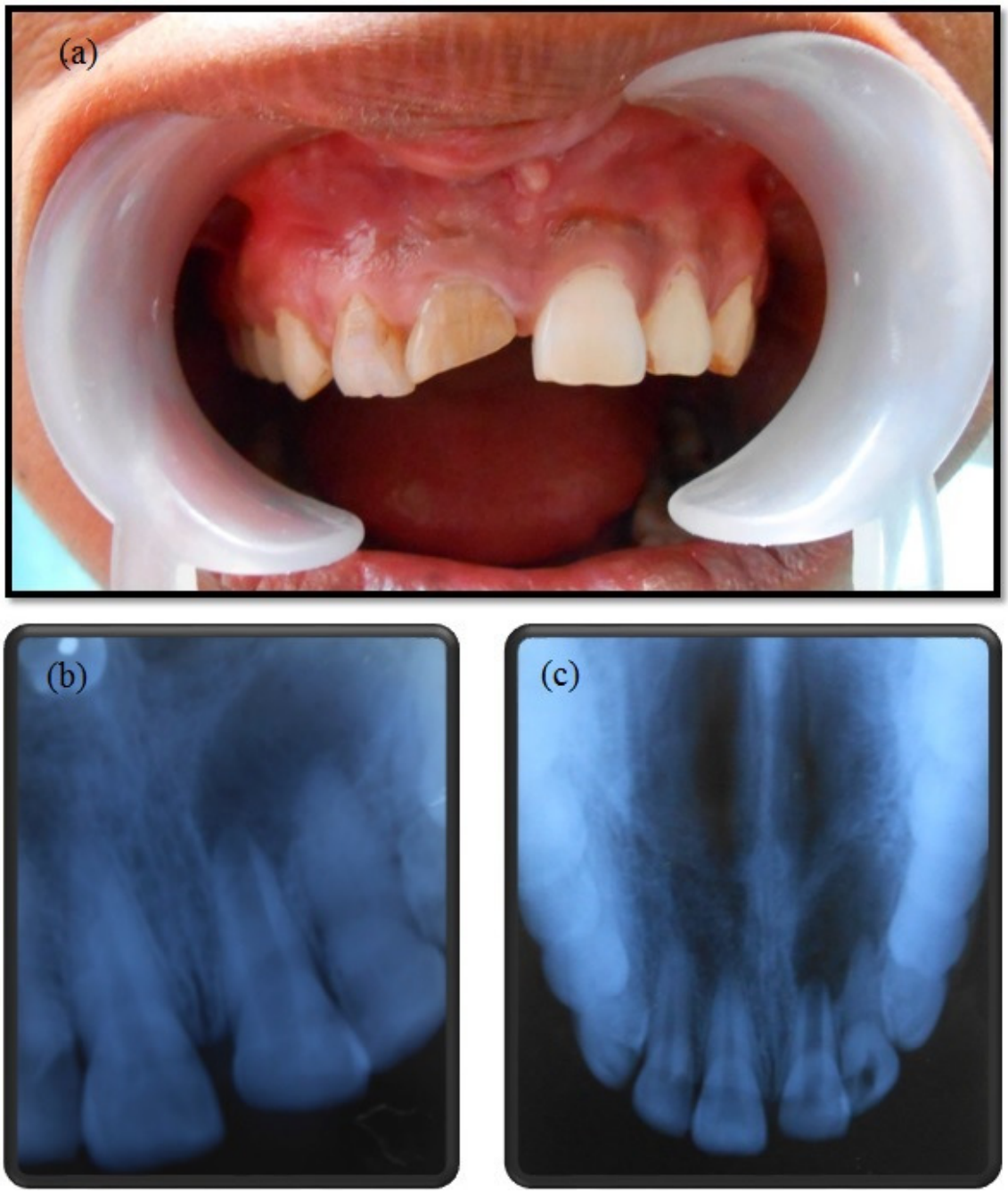

Fig. 1. Pre-operative views of case a) clinical view showing discoloured and fractured right maxillary central incisor, b) Intra-oral periapical (IOPA) radiograph, c) occlusal view showing radiolucency in relation to right maxillary central, lateral incisors and calcified canal of lateral incisor

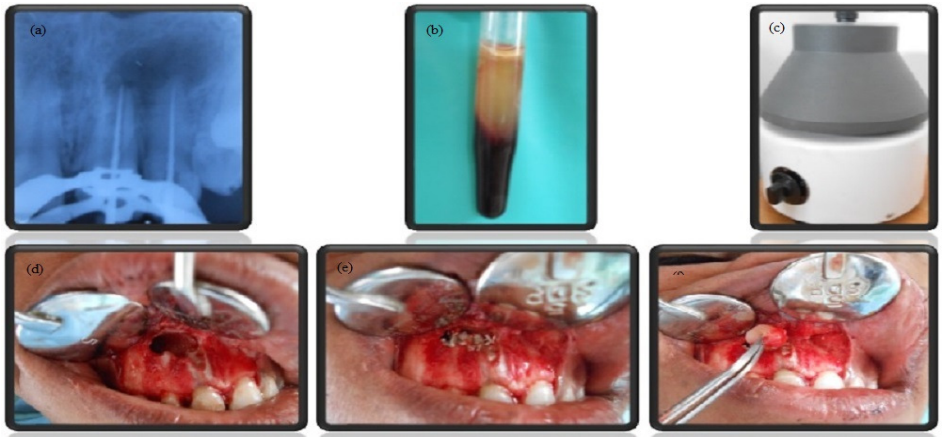

Fig. 2. a) IOPA radiograph showing negotiation of calcified canal of right maxillary lateral incisor, b) three layers of blood clot after centrifugation: a base of RBCs, at the bottom, acellular plasma on the surface, and platelet rich fibrin clot in the middle, c) centrifuge used at 3000rpm for 10mins, d) debridement of bony cavity after flap retraction, e) placement of bone graft, f) placement of platelet rich fibrin

Fig. 3. Post-operative a) Immediate post-operative IOPA radiograph, b) IOPA radiograph after 3months follow up showing some amount of healing, c) IOPA radiograph after 6months follow up showing more than 90% healing and presence of bony trabeculae, d) clinical picture after 6 months follow up showing healthy gingival margins and no signs of sinus or oedema

3. DISCUSSION

Repair and regeneration is the key to success for endodontic therapy. Regeneration of tissue after a surgical procedure requires (a) recruitment of progenitor/stem cells to differentiate into committed cells, (b) growth/differentiation factors as necessary signals for attachment, migration, proliferation and differentiation of cells, and (c) local-microenvironmental cues like adhesion molecules, extracellular matrix, associated non-collagenous protein molecules, and so forth. Lack of any of these elements would result in repair rather than regeneration [16].

Commonly used method for regeneration in periapical surgery is bone replacement grafts. These grafts can promote tissue or bone regeneration through variety of mechanisms. Bone grafting materials include autografts, allograft, xenografts, and alloplasts. Alloplasts such as osteoconductive calcium phosphate have been widely used in periapical surgery to enhance new bone formation [17]. Several case reports have demonstrated healing with mature bone and haemopoietic marrow in periapical areas by using this bone graft [18-20].

The use of PRF is a recent and promising innovation in periodontal regenerative therapy. The positive impact of PRF on bone healing could be attributed to the angiogenic, proliferative and differentiating effects on osteoblasts of tissue growth factor β and platelet derived growth factor (TGF-β and PDGF) that are present in PRF in high concentrations [21]. PDGFs comprise a family of homo- or heterodimeric growth factors, including PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD. They exert their functions by binding to three different transmembrane tyrosine kinase receptors, which are homo- or heterodimers of an α- and a β-chain [22,23]. PDGF was the first growth factor shown to be chemotactic for cells migrating into the healing skin wound, such as neutrophils, monocytes, and fibroblasts. In addition, it enhances proliferation of fibroblasts and production of extracellular matrix by these cells. Finally, it stimulates fibroblasts to contract collagen matrices and induces the myofibroblast phenotype in these cells [23,24]. Thus it has long been suggested to be a major player in wound healing. Indeed, a series of experimental and clinical studies have demonstrated a beneficial effect of PDGF for the treatment of wound healing disorders [23].

PRF has also been successfully used with different graft materials, with and without GTR, in the treatment of human periodontal infrabony defects [25-28].

In present case combination of nanocrystalline hydroxyapatite with collagen and PRF is used, as it helps in faster bone regeneration. Graft material is osteoconductive and collagen network provides a better scaffold for clot formation and bone regeneration. Osteoconduction is a process wherein the bone is formed by the in-growth of capillaries and osteoprogenitor cells from the recipient bed into, around, and through a graft. These Hydroxyapatite crystals when used act as a scaffold upon which new bone is deposited, which is then followed by a slow resorption of the graft. PRF is used, as it helps in better healing and faster regeneration, because of the biological modulators and also helps in osteoinduction. PRF acts as a biological connector between different elements of graft while acting as a matrix favouring neoangiogenesis, capture of stem cells and migration of osteoprogenitor cells to the center of the graft [29].

However, like other clinical studies this study also has few limitations like short follow-up period of 6 months and a need for histological evaluation to confirm regeneration.

4. CONCLUSION

The combination of PRF and nanocrystalline hydroxyapatite with collagen has been demonstrated to be an effective approach to induce faster periapical healing in present case with large periapical lesion. The precise mechanism of action of PRF is yet to be proved. PRF is a second generation platelet concentrate which is still under study and many more advancements in its clinical applications are expected in near future. This case report is beginning of a larger clinical project.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

An ethical approval was obtained from institution's ethical committee to carry out surgical procedure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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