CASE REPORT

# CBCT EVALUATION OF HEALING OF BONY DEFECTS IN AN APICOECTOMISED TOOTH USING HYDROXYAPATITE CRYSTALS, PLATELET RICH FIBRIN AND BARRIER MEMBRANE : A CASE REPORT

Dr. Shukdeb Mandal*, Dr. H.D Adhikari**, Dr. Parthasarathi Mondal***
Dr. Pampa Adhya***, Dr. Siddhartha Das****

## ABSTRACT

Healing of large bony crypt after periapical surgery by regeneration is a challenge for endodontists. Fibroblasts compete with osteoblasts and healing takes place mostly by fibrous tissue. This is not desirable. Osteoconductive & osteoinductive graft materials and a barrier membrane have been used by some researchers for bone regeneration. But the literature is scanty in this field. In the management of present case, the large periapical bony cavity (10 x 11mm) in relation to 21 & 22 was filled up with Hydroxyapatite (HA) crystals and autologous Platelet Rich Fibrin (PRF) mixture and the apicomarginal bony defect over the labial surface of latter as well as the graft material were then covered with Guided Tissue barrier membrane after apicoectomy. IOPAR & CBCT evaluation upto 12 months revealed the evidence of gradual healing of the bony crypt and the apicomarginal defect by regeneration of bone tissue. The result of the present case report helps to explain the successful use of HA & PRF mixture as a graft material and a GTR membrane in regeneration of bone in large periapical bony crypt with apicomarginal defect after apicoectomy.

## KEY WORDS

**PRF, HA, Guided Tissue barrier membrane, apicomarginal defect, apicoectomy, CBCT, regeneration.**

## ABOUT THE AUTHORS

*Final year Postgraduate student
**Professor and Head
***Associate Professor
****Assistant Professor
Dept. of Conservative Dentistry and Endodontics
Dr. R. Ahmed Dental College and Hospital, Kolkata

**CORRESPONDING AUTHOR**
**Dr. Shukdeb Mandal**
Final year Postgraduate student
Dept. of Conservative Dentistry and Endodontics, RADCH
e-mail address:- dr.sdebmandal@gmail.com
Contact number:- 9830550139

## INTRODUCTION

Regeneration of bone in periapical bone defects in apicoectomised tooth is a great challenge to endodontist, especially in case of large or through and through lesions. The nature of wound healing may be repair or regeneration depending on the nature of wound ; the availability of progenitor cells; signaling molecules; and micro-environmental cues such as adhesion molecules, extracellular matrix, and associated non-collagenous protein molecules.[1] Regeneration is defined as the reproduction or reconstitution of a lost or injured part of the body in such a way that the architecture and function of the lost or injured tissues are completely restored.[2] Since repair is not desirable, regenerative approaches that aim to restore the lost tissues such as periodontal ligament, bone, cementum, and connective tissue, have been introduced.[3] It frequently includes the use of bone grafting materials and barrier membrane to encourage the growth of these surrounding tissue, while excluding unwanted cell types such as epithelial cells or fibroblasts.[4]

Porous hydroxyapatite (HA) has been used to fill the periodontal intrabony defects, which has resulted in clinically acceptable responses.[5] It has been shown that porous HA bone grafts have excellent bone conductive properties, which permit outgrowth of osteogenic cells from existing bone surfaces into the adjacent bone defect.[6]

Platelet-rich fibrin (PRF) is a second-generation platelet concentrate which is a rich source of various growth factors and cytokines embedded within the fibrin clot.[7] PRF is osteoinductive, and HA is osteoconductive. This combination offers synergistic effect on bone regeneration. Clinical data reveal that it will be a favorable matrix for excellent healing without excess inflammation[8] because of several advantages[9].

I. Fibrin clot provides mechanical and biological connector between HA particles

ii. This integration of fibrin net promotes various stages of healing such as cellular migration, especially endothelial cells for the purpose of neoangiogenesis, vascularization and graft survival.

iii. As the fibrin matrix is resorbed in passage of

time, growth factors are gradually released and healing process continuously progresses.

iv. Cytokines and incorporated leukocytes play significant role in controlling inflammation within the graft material[10].

Guided tissue regeneration therapy using GTR membrane or barrier membrane introduced in 1980s has been widely used to regenerate lost periodontium from periodontal disease. Scientific evidences indicate that principles of GTR can be successfully applied in endodontic surgery to correct bone defects confined to periapical region. The placement of GTR membrane over an osseous defect can prevent the faster proliferating oral epithelium and gingival connective tissue from growing into bone defect, allowing the cells of the periodontal ligament and endosteum to colonize and regenerate the lost tissue.[11]

Study of Sculean et al.[12] revealed that the combination of barrier membrane and graft materials may result in histological evidence of periodontal regeneration, predominantly bone repair. Pradeep et al.[13] in their study showed that HA and PRF mixture increased the regenerative effects in the treatment of three wall intrabony defects. But, clinical study in the context of periapical surgery is sparse. Therefore, the purpose of this case report is to addknowledge to the existing literature about the combined use of graft material and barrier membrane in the treatment of a large periapical lesion with dehiscence.

## CASE REPORT

A 19 years old female patient was referred from Hospital OPD to this department for treatment of 21 and 22. The patient presented with pain and swelling, and gave a history of dental trauma 7 years back. Patient's medical history was non-contributory. Clinically, there was presence of discoloration of 21 and discharge of pus on pressure from buccal periodontal sulcus of 22. IOPAR revealed a large periapical radiolucency (about 10 mm X 11 mm) in relation to both the teeth (Fig. 1). CBCT scan was performed to evaluate the periapical lesion quantitatively. (Fig.-2; Table I)

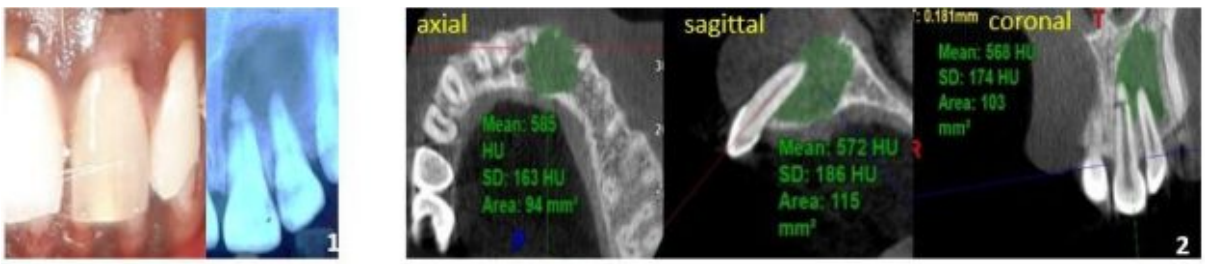
## MATERIALS AND METHODOLOGY

RCT followed by periapical surgery was planned and explained to the patient. An informed consent was obtained. Clearance from Instituitional Ethics Committee was taken. Routine blood investigations were advised and the reports were found within normal limit.

Maintaining the standard protocol, root canal treatment was performed on both the teeth and access cavities were double sealed by Resin Modified Glass Ionomer Cement and composite resin. Periapical surgery was planned thereafter.

Under local anaesthesia (2% Lignocaine with 1:80,000 adrenaline) a crevicular and two vertical releasing incisions were given (Fig.-4) and a mucoperiosteal flap in relation to 11,21,22,23 was raised revealing apicomarginal defect of the buccal cortical bone along the root surface of 22 (Fig.-5). A bony window was made labially with a no. 6 round TC bur along with copious irrigation using sterile saline solution. Apical 3 mm of roots of 21 and 22 were removed using tapered fissure bur along with en masse curettage of the apical pathology and integrity of the palatal periosteum was checked. 0◦ angulation of resected root ends wasensured (Fig.-6). The resected tissue was sent for histopathological evaluation. Retrograde cavities of 3 mm depth were prepared with the help of ultrasonic tip (Fig.-7 and Fig.-8) and retrograde fillings were done in both 21 & 22 with wMTA (Pro Root, Dentsply, Tulsa, USA) (Fig.- 9 and Fig.-10).

Then, preparation of Platelet Rich Fibrin (PRF) was done in accordance with the protocol set by Chukroun et al.[14] 10 ml blood was drawn from patient's antecubital vein (Fig.-11). It was immediately transferred in a sterile test tube without an anticoagulant and centrifuged at 3000 rpm for 10 mins in a table top centrifuge machine (REMI Model R-8C, India) (Fig.-12). After centrifugation, PRF was separated from Platelet Poor Plasma at the top and RBC at the bottom of the test tube (Fig.-13). PRF was taken out with the help of a sterile tweezer (Fig,14). Bulk of leucocyte layer was removed with the help of sterile scissor and a layer of 1-2mm of it was allowed to remain along with the PRF. It was then mixed with




**Pre-op evaluation: Fig. 1- Intra-oral pic & IOPAR. Fig. 2- CBCT scan showing destruction of buccal cortical bone in relation to pathology, perforation of palatal cortex and loss of bone over root of 22**

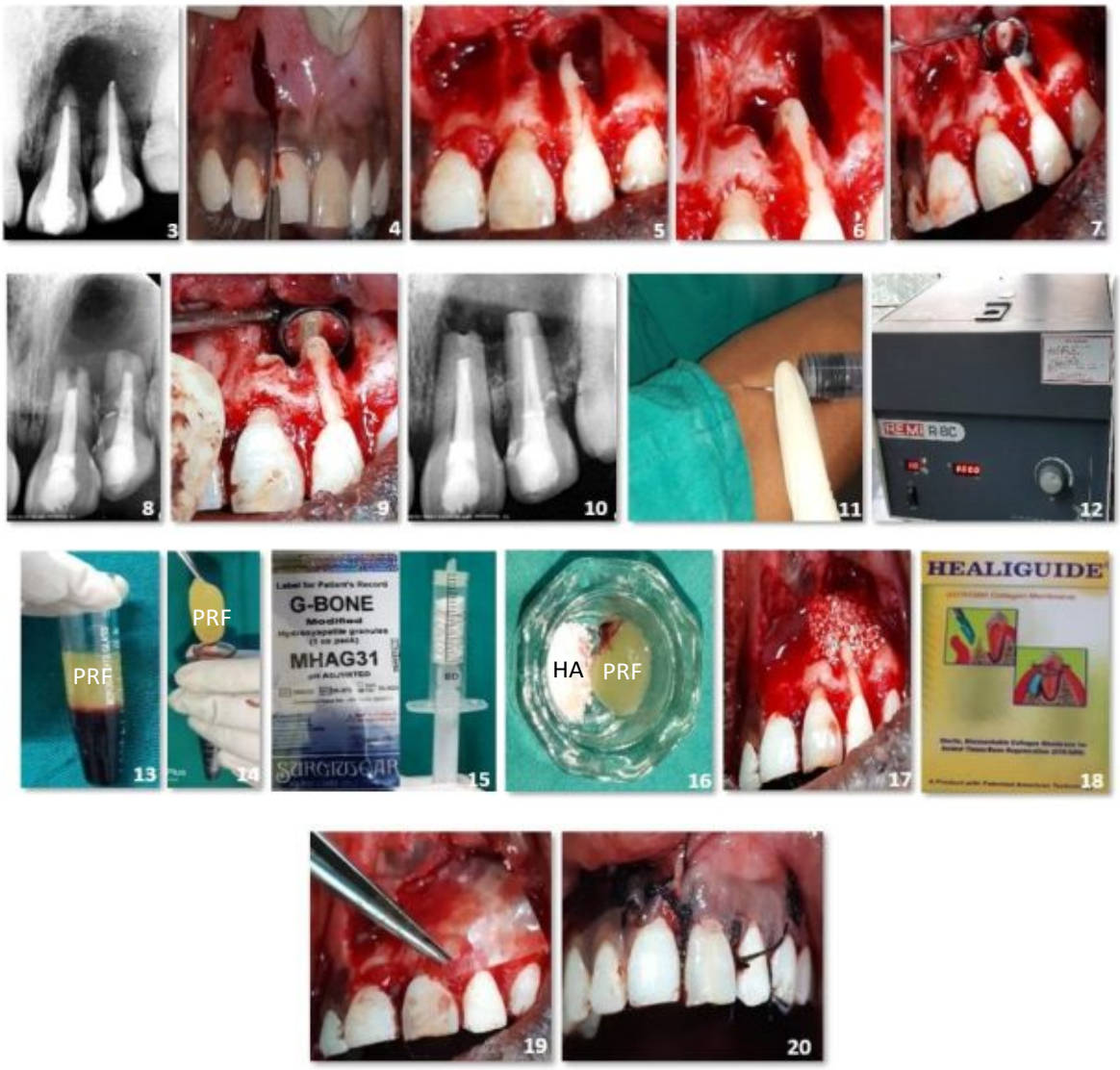
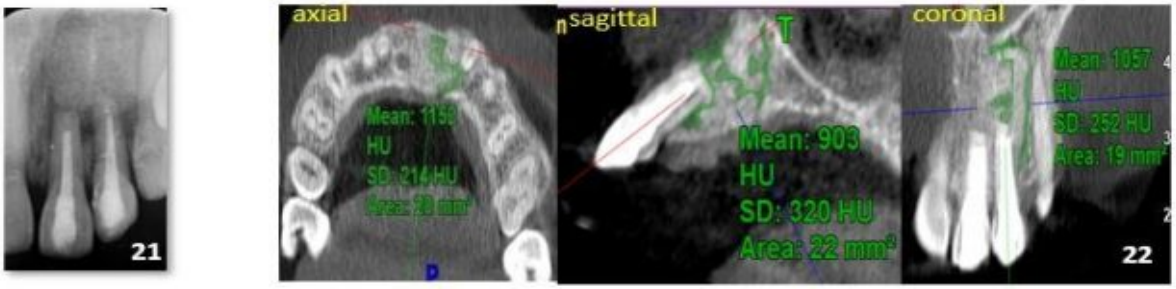
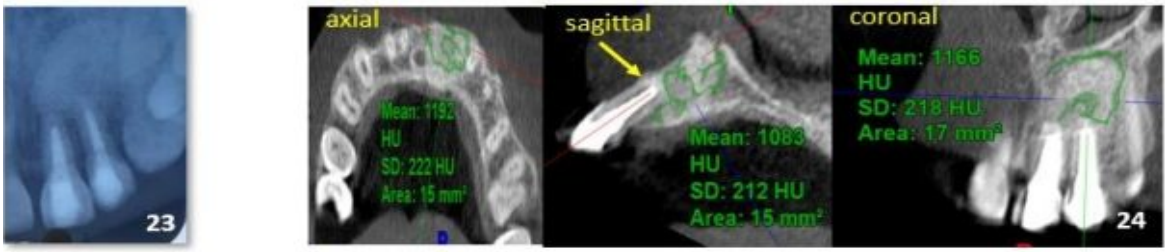


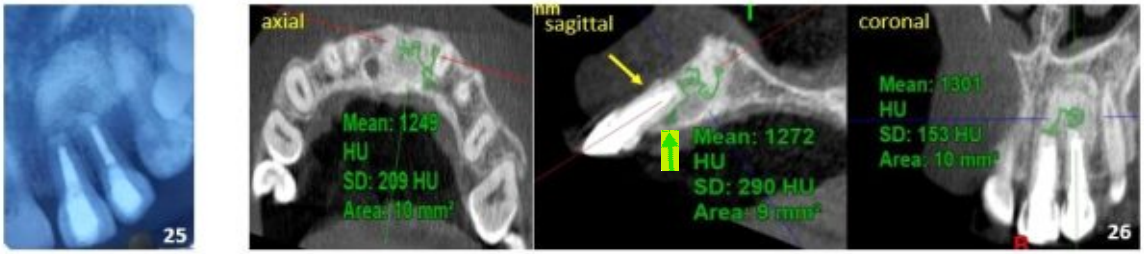

**Fig.3 – After obturation and Composite restoration. Fig.4 – Oblique vertical incision. Fig.5 – Surgical site exposed, apicomarginal defect 22. Fig. 6 -Root Resected. Fig.7 & Fig. 8 – Rootend cavity. Fig.9 & Fig.10 - Showing retrocavity filling with MTA. Fig.11- Blood drawn from patient's antecubital vein. Fig.12 – Blood centrifuge machine. Fig.13- PRF at the bottom of the test tube. Fig.14 - PRF taken from test tube. Fig.16- PRF & HA in a sterile dappen dish. Fig.15- Bone graft material; HA crystals. Fig.17- Filled with PRF & HA crystals mixture. Fig.18- GTR Membrane. Fig.19 - Placement of GTR membrane. Fig.20- Sutures given.**




**3 mon. post-op: Fig.21 – IOPA R; Fig.22 - CBCT showing increased bone density and decrease in surface area of the bony cavity.**




**6 mon. post-op: Fig.23 – IOPA R; Fig.24 - CBCT showing further increase in bone density, decrease in surface area of the bony cavity. Evidence of bone formation labial to 22 in sagittal view (arrow) as well as in palatal cortex repairing the perforation.**




**12 mon. post-op: Fig.25 – IOPA R; Fig.26 - CBCT showing further increase in bone density, decrease in surface area of the bony cavity, progressive bone formation labially (arrow) and normal configuration of the palatal cortex in relation to 22 in Sagittal view(arrow)**

**TABLE I:**
**INCREASE IN BONE DENSITY (HU) AND REDUCTION IN AREA (A) OF BONY DEFECT (mm2)**

| TIME PERIOD | | CBCT SCAN | | | AVERAGE VALUE | % Gain in HU | % Red.in A Preop-Fl up/preop x 100 |
|---|---|---|---|---|---|---|---|
| | | AXIAL | SAGITTAL | CORONAL | | | |
| Pre-op | HU | 585 | 572 | 599 | **585.3** | | |
| | A | 94 | 115 | 110 | **106.3** | | |
| 3 mon. | HU | 1152 | 903 | 1057 | **1037.3** | **77.2** | |
| | A | 20 | 22 | 19 | **20.3** | | **80.9** |
| 6 mon. | HU | 1192 | 1083 | 1166 | **1147** | **95.9** | |
| | A | 15 | 15 | 17 | **15.6** | | **85.2** |
| 12 mon | HU | 1249 | 1272 | 1301 | **1274** | **117.6** | |
| | A | 10 | 09 | 10 | **9.6** | | **90.9** |

HA bone graft (G-BONE, INDIA) (Fig. -15) in a sterile dappen dish (Fig.-16) and the composite graft was then placed into the periapical defect (Fig.- 17).

GTR membrane (15mmX20mm) (HEALIGUIDE, Advanced Biotech Products (P) Ltd. Tamil Nadu, INDIA) (Fig.-18) was then placed over the osseous defect (Fig.-19). The size of membrane was trimmed in such a manner that it covered the bony crypt and the apicomarginal defect 2-3 mm beyond the margin. Amount of the graft material was sufficient enough, so that the membrane did not collapse. After stabilizing, the flap was sutured using 3-0 silk (Fig.-20). Antibiotics and analgesics were prescribed. The patient was disposed with proper advice and asked to report after 7 days for suture removal. After suture removal, she was adviced for recall visits.

On follow-up visits, patient was evaluated clinically and radiologically. There were no symptoms of inflammation, pain, swelling or discomfort and the teeth were functioningnormally. On periodontal probing, gingival sulcus depth was found normal in both the teeth. Evidences of bony healing were observed in 3, 6, and 12 months radiograph (fig: 21-fig:26) within the crypt and also on labial surface of 22 as it was reflected in sagittal view of CBCT from 6 month onwards. The increase in bone density expressed in HU and decrease in area in mm2, of the bony defect viewed in CBCT scan from the baseline (pre-operative) onwards upto last follow-up visit was evaluated by % increase in HU and % decrease in A (Table:1)

## RESULTS

From TABLE I, it appears that bone density of the defect has increased to 77% in 3 months, 99.5% in 6 months and 117.6% in 12 months follow up visit and the gradual reduction of surface area of the defect has also been observed which was 80.9%, 85.2% and 90.9% in the respective follow up periods.

## DISCUSSION

The early bone regeneration in apicoectomised tooth with large periapical lesion is required to provide functional support to the tooth. If required, orthodontic treatment can also be done only after the healing of the wound.

The four critical factors that influence bone regeneration after the periapical surgery are primary wound closure, angiogenesis for blood supply and source of undifferentiated mesenchymal cells, space maintenance, and stability of the wound (PASS principle)[15].

PRF in the form of platelet gel improves the handling properties of graft materials, helps in graft stabilization, wound closure, haemostasis, and maintenance of the space of bone crypt to be replaced by bone tissue.[16]

Clinical trials suggest that the combination of bone graft along with the growth factors in the PRF may be suitable to enhance the bone density.[16] PRF is a rich source of platelet derived growth factor (PDGF), transforming growth factor β (TGF-β), insulin like growth factor (IGF), vascular endothelial growth factor (VEGF), Epidermal Growth Factor (EGF) etc.

PDGF - promotes angiogenesis, activates macrophages that initiate the release of growth factors from host tissue which enhances bone, cementum, and periodontal ligament repair and regeneration.[17,18]

TGF-β activates fibroblasts to induce collagen (type-I) formation, endothelial cells for angiogenesis, chondroprogenitor cells for cartilage and mesenchymal cells to increase the population of wound healing cells.[19]

IGF-I stimulates bone formation by proliferation and differentiation[20], and it is synthesized and secreted by osteoblasts.[21]

VEGF also promotes angiogenesis, increases vascular permeability, stimulates mitogenesis for endothelial cells .[31]

EGF stimulates endothelial chemotaxis /angiogenesis, regulates collagenase secretion and epilthelial /mesenchymal mitogenesis. [31]

An increase in the proliferation of human osteoblasts has been demonstrated with a combination of PDGF, IGF-I, TGF, and Epidermal Growth Factor (EGF).[22]

In this case report, hydroxyapatite crystals with average granule size of 0.5-0.9 mm were used. Nearly, same granule size (0.5-1mm) of hydroxyapatite was used by Sreedevi PV et al. (2011)[23] to fill the osseous defects following periapical surgery.

Basal cells of oral epithelium are attached to basement membrane (BM) through hemidesmosomal attachment. These cells proliferate due to inflammation and benign hyperplasia. BM prevents the passage of these cells to deeper layer of connective tissue. However, malignant epithelial cells produce proteolytic enzymes like matrix metalloproteinases, collagenases which destroy the BM comprising of basal lamina (laminin, Type IV collagen) and reticular lamina (Type III collagen) and then invade the underlying connective tissue.[24] So, epithelial cells in the mucoperiosteal flap in periapical surgery are not capable to penetrate into the bony defect even when barrier membrane is not used.It is generally believed that fibroblasts move faster than osteoblasts to occupy the bony defect left after apical surgery and thus consequently, a scar tissue is formed in large bone defects.[25,26] Barrier membranes are thought to prevent movement of proliferating fibroblasts from the surrounding tissue into the bony defect.[27,25,26] The movement of cells is a biologic process and complex in nature. It does not depend on cell mobility only. Cell surface integrins, extracellular matrix molecules, and concentration of chemokines and growth factors control cell movement.[28] Importantly, during periapical bone wound healing after apical surgery, the newly differentiated osteoblasts are derived from bone marrow mesenchymal stem cells and osteoprogenitor cells lining the endosteum.[29] These osteoblasts do not have to compete with fibroblasts from surrounding tissue to occupy the surgical bony crypt. This allows regenerative cells of bone, PDL, cementoblast to repopulate the area.[3]

Animal study[30] also showed complete bone filling of periapical bone cavities after endodontic surgery, only when a barrier for guided tissue regeneration was used, whereas extensive connective tissue filling of the defects was found after conventional endodontic surgery.

The present case in this article was a complicated one. There was destruction of labial cortical bone in relation to the pathology involving two incisors on left side with the apicomarginal defect on the labial surface of the lateral incisor with discharging pus through the deep periodontal pocket. Also, there was expansion of palatal bone with perforation. In the management of this case, HA and PRF mixture and GTR membrane were used. Satisfactory result was observed after follow-up period of one year only.

## CONCLUSION

Regeneration of bone in bony crypt after apicoectomy and in the apicomarginal defect can be achieved by using HA crystals, PRF, and GTR membrane.

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