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CBCT Evaluation Of Bony Healing In Apicoectomized Tooth Using Sticky Bone With GTR And PRF Membrane As Barrier Membrane: A CASE SERIES

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Abstract:
Background*: This Clinical Work Was Conducted On Two Cases To Evaluate The Healing Of Bony Periapical Lesions After Surgical Endodontics.*
Materials And Methods*: Conventional Endodontic Treatment Followed By Surgical Endodontic Treatment Was Performed By Confirming Anatomical Configuration Of Bony Defects Like Dehiscence, Before And After Treatment Procedure With A Cone Beam Computed Tomography (CBCT) Image Analysis.*
Results*: It Was Observed That There Was Gradual Increase In Bone Density And Reduction In Surface Area Of Radiolucent Area Over 6 Month Follow-Up In Both The Cases.*
Conclusion: *Sticky Bone In Combination Along With Resorbable GTR Membrane And PRF Membrane Was Seen To Be Promising In Inducing And Accelerating Hard And Soft Tissue Regeneration.*
Key Word*: Dehiscence, CBCT, Apicoectomy, Guided Tissue Regeneration (GTR), Regeneration, Platelet-Rich Fibrin, Surgical Endodontics.*

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I. Introduction

Healing by regeneration of bony defect in periapical region after surgical endodontics is a challenge to endodontists. After endodontic surgery healing of periapical tissues occurs by repair or regeneration depending on the nature of the wound; the availability of progenitor cells; signaling molecules; and micro-environmental molecules such as adhesion molecules, extracellular matrix, and associated non-collagenous proteinmolecules.[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]

Repair is defined as the healing of a wound by tissue that does not fully restore the architecture or the function of the part. Since, repair is not an ideal outcome of wound healing, newer approaches such as regenerative procedures aimed at restoring lost tissues, have been introduced.[3]

The bone regeneration following periapical surgery can be facilitated by placing bone graft into the periapical defect to act as a mechanical substructure that supports the membrane (Guided Tissue Regeneration Membrane) and the overlying soft tissues and to serveas a biologic component that enhances bone formation.[4]

Guided tissue regeneration (GTR) techniques have been widely used for bone and periodontal tissue regeneration. In endodontic surgery, GTR has been applied using different bone substitute materials and/or different barrier membranes based on the concept that if epithelial cells, that migrate approximately ten times faster than other periodontal cell types [Engler et al.(1966)] are excluded from the wound space long period; epithelial downgrowth is prevented and regeneration can be achieved by other cell types like osteoblasts with regenerative potential.

Plasma concentrates like Platelet rich fibrin (PRF) is a second generation platelet concentrate, has a three-dimensional architecture conductive with stem cell proliferation and differentiation andcontains bioactive molecules.[5]

PRF membrane is a autologous, bioactive membrane which is a three-dimensional matrix of platelets, leukocytes, and growth factors. When compression is applied on PRF gel, it becomes a membrane like form which can be used in endodontic surgical procedure for Guided tissue regeneration (GTR) techniques.

Sticky Bone is a growth factors-enriched bone graft matrix, which is prepared by preparing Autologous Fibrin Glue (AFG) from patient's own blood and then mixed with particulate bone powder and allowed to rest for 5-10 min for polymerization, which results in a yellow coloured mass known as sticky bone. This combination resulted in greater pocket depth reduction, defect fill and gain clinically than PRF used alone. Hydroxyapatite(HA) could maintain the space for tissue regeneration and it could enhance the effects of plasma concentrates.

GTR membranes are cell-proof barriers against soft tissue invagination, whereas PRF membranes allow cells to migrate through it, thus allowing new blood vessel formation that will facilitate regenerative and healing interactions between the tissues below and above the PRF membrane. The PRF membrane is a highly stimulating matrix, attracting cell migration and differentiation preferentially, and also reinforcing the natural periosteal barrier.

Sculean et al.(2008)[6] revealed that the combination of barrier membrane and graft materials may result in histological evidences of periodontal regeneration, predominantly bone repair. Pradeep et al.(2012)[7] showed in their study that HA and PRF mixture increases the regenerative effects in the treatment of three wall infrabony defects. Therefore, the purpose of this case report is to add knowledge to the existing literature about the use of bone graft like sticky bone with barrier membrane, GTR and PRF membrane in the treatment of large periapical lesion with dehiscence.

II. CASE REPORTS:

CASE 1 : Surgical endodontic procedure using sticky bone and PRF membrane:

A 24yrs old male patient was referred from the outpatient department to the department of conservative dentistry and endodontics of Dr. R. Ahmed dental college and hospital, Kolkata for treatment of 21, 22 & 23. The patient presented with pain and swelling, and gave a history of dental trauma in his childhood. Patient's medical history was non-contributary. Clinically, there was presence of discoloration of 21 and pus discharge on pressure from labial periodontal sulcus of 22. IOPAR revealed a large periapical radioluscency (about 11mm x 12mm) in relation to the perapical region of all three roots (Fig.1). CBCT scan was performed to evaluate the periapical lesion quantitavely (Fig.2).

Fig. 1: PRE-OP CLINICAL PICTURES AND RADIOGRAPHS

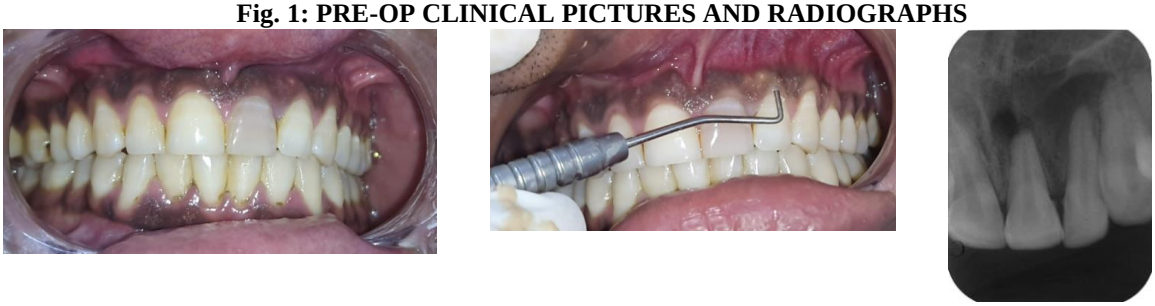
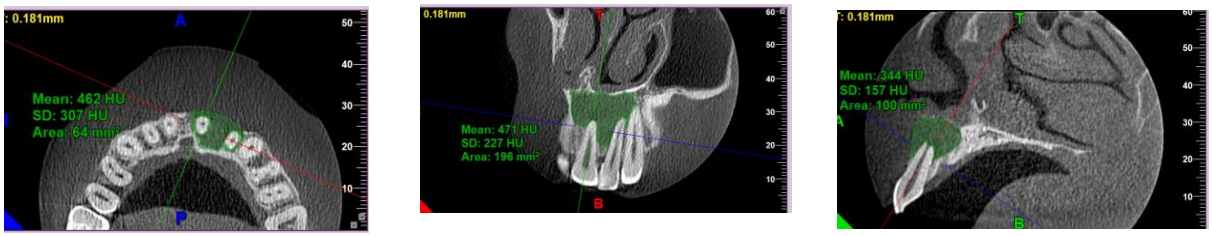


Fig. 2: PRE-OP CBCT



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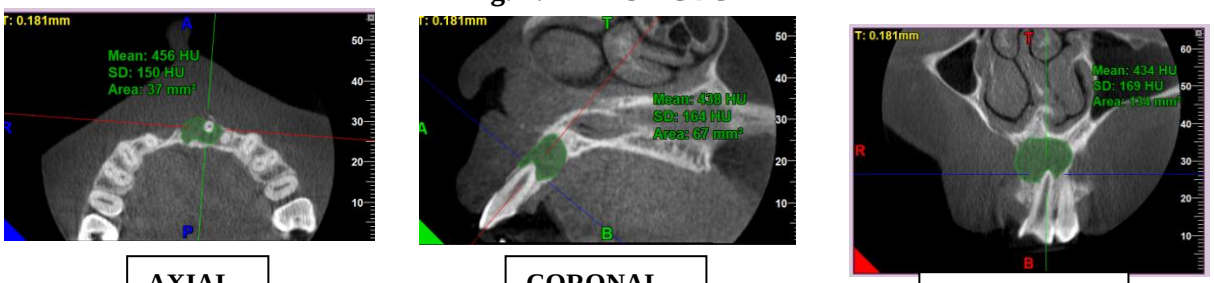
CASE 2 : Surgical endodontic procedure using sticky bone and GTR membrane:

A 27yrs old female patient was referred to the department for treatment of 21 & 22. Patient complained of pain and swelling with a history of dental trauma 7 years back. Patient had no contributory medical history. Clinically, 11 was missing due to avulsion caused by dental trauma and discoloration of 21 with discharge of pus from gingival sulcus. IOPAR revealed large radioluscency (6mm x 7mm) at the apical region of both the teeth roots (Fig.3). CBCT scan was performed to evaluate the periapical lesion quantitavely (Fig.4).

Fig. 3: PRE-OP CLINICAL PICTURES AND RADIOGRAPHS

Fig. 4: PRE-OP CBCT



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III. Material And Methods

Conventional endodontic treatment followed by periapical endodontic surgery was planned which was explained to the patient and an informed consent was taken. Clearance from Institutional Ethical Committee was taken. Routine blood investigations were advised and the reports were found within normal limit.

Maintaining standard protocol, root canal treatment was performed on the involved teeth of selected cases. The access cavity was double sealed by Resin Modified Glass Ionomer Cement (RMGIC; GC Gold Label 2 LC) and composite restoration (Te-Econom Plus composite, Ivoclar). Thereafter, periapical endodontic surgery was planned.

Under local anaesthesia (2% Lignocaine with 1:80,000 adrenaline) a crevicular and two vertical release incision were given (Fig.5 & 7) and the mucuperiosteal flap was raised. A bony window was prepared labially with no. 6 round TC bur along with copious irrigation using sterile saline solution. Apical 3mm of root ends of the involved teeth was resected using tapered fissure bur along with enmass curettage of periapical pathology and integrity of plalatal periosteum was checked. 0° angulation was maintained while root resection and the removed periapical specimens were sent for histopathological examination. Retrograde cavity of 3mm depth was prepared using ultrasonic retro-tips and filled with wMTA (Pro Root, Densply, Tulsa,USA).

The sticky bone was prepared by withdrawing 20 ml of blood in non-coated sterile test tube which is then centrifuged at 2400-2700 rpm for 2 min. The deeper layer is RBC's and the superficial layer is AFG. This AFG is then extracted using a syringe and mixed with particulate bone powder and allowed to rest for 5-10 min for polymerization, which results in a yellow coloured mass known as sticky bone. The sticky bone thus prepared is used as graft material to be used and filled into the bony cavity.

The PRF was prepared following Dohan's protocol. From the patient's antecubital vein, 10 ml of blood was drawn and transferred into a sterile test tube without anticoagulant. It was then immediately centrifuged in a centrifugation machine at 2700 rpm for 12 mins. At the end of the process a structured fibrin clot, formed in the middle just between platelet-poor plasma(PPP) at the top and the red corpuscles at the bottom, was obtained. PRF was separated from red corpuscles base using sterile tweezers just after removal of PPP and then transferred into a sterile dappen dish. The PRF clot is compressed to form membrane by placing the PRF clot in the L-PRF Wound Box. Therefore, the prepared PRF membrane is used as barrier membrane and placed on to the bone graft placed into the bony defect and cavity in case 1(Fig.5) and in case 2, GTR membrane (HEALIGUIDE, Advanced Botech Products(P) Ltd. Tamil Nadu,INDIA) was placed over osseous defect (Fig.7) as barrier membrane.

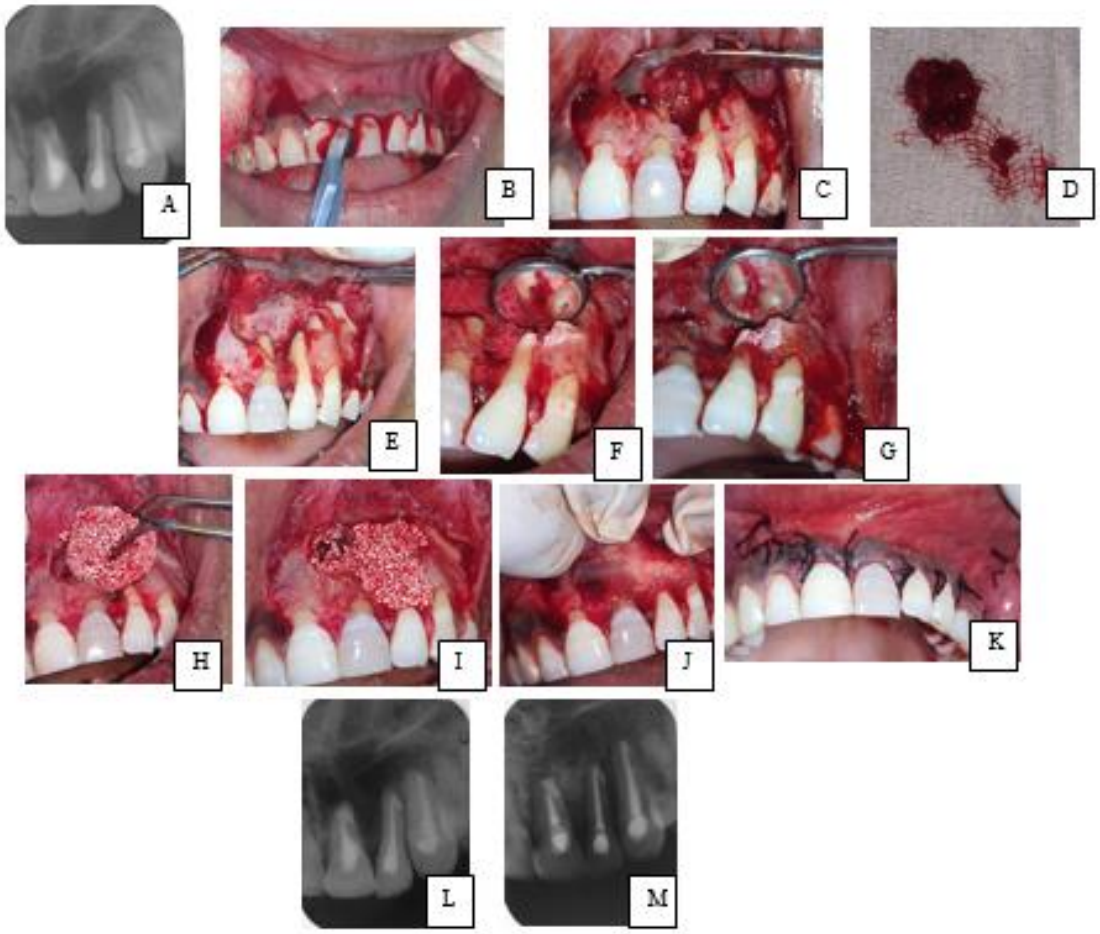
The surgical site was checked for proper placement of bone graft and barrier membrane followed by repositioning and stabilization of mucoperiosteal flap, suture was given using 3-0 silk (Fig. 5&7). Antibiotics and analgesics were prescribed. Both the patient were discharged with proper advice and asked to report after 7 days for suture removal. After suture removal, patients were advised for recall visits for follow-up.

On follow-up visits, patients were evaluated both clinically and radiologically. There were no symptoms of inflammation, pain, swelling or discomfort and teeth were functioning within normal limits. On periodontal probing, gingival sulcus depth was found normal. Evidence of bony healing was observed and compared at 6 month with that of pre-op radiographically with help of CBCT analysis(Table-1).

Table-1:Increase in bone density(HU),reduction in area(A) of bony defect(mm^2)

Case No.	Time period		CBCT Scan			Average value	% gain in HU	% Red. of area
			Axial	Coronal	Saggital			
Case No.1	Pre-op	HU	462	471	157	363	57.20	75.3
		A	64	196	100	120		
	6 mon.	HU	1365	601	579	848.3		
		A	4	58	27	29.6		
Case No.2	Pre-op	HU	456	434	438	442.6	51.28	81.9
		A	37	134	67	79.3		
	6 mon.	HU	1064	1051	611	908.6		
		A	6	5	10	14.3		

Fig. 5: Case No.-1: Surgical steps



A. Post-obturation IOPAR, B. Incision placed, C. Mucoperiosteal flap raised, D. Resected cystic lining, E. Bony cavity, F. Retro cavity prepared, G. MTA filled retro cavity, H. Prepared sticky bone carried to bony cavity, I. Sticky bone placed into bony cavity, J. PRF membrane placed, K. Suture placed, L. Immediate post-op, M. 6 month IOPAR

Fig. 6: POST-OP CBCT(6 months follow-up)

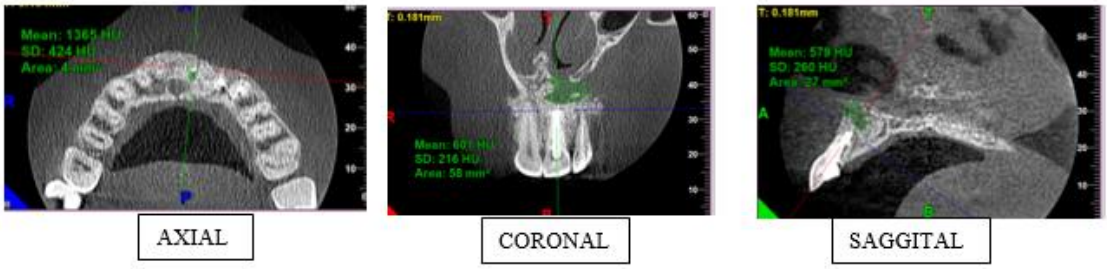
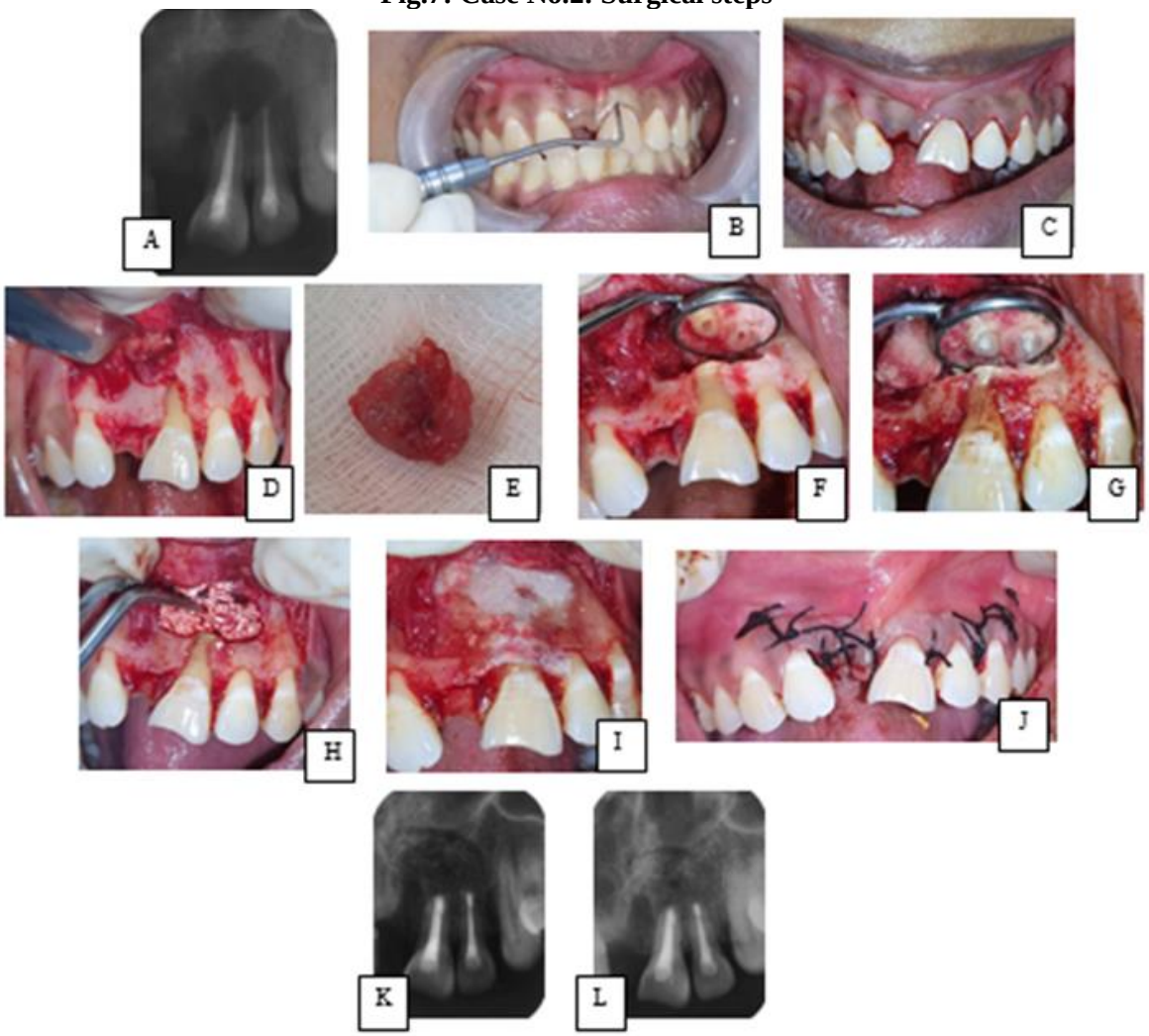
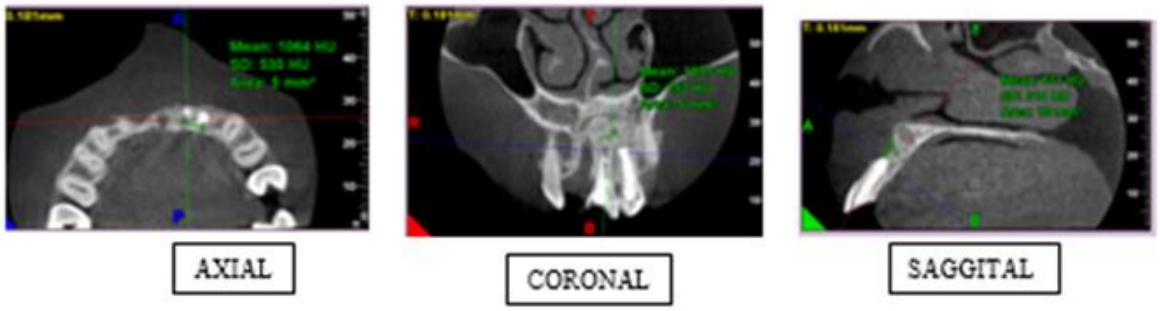


Fig.7: Case No.2: Surgical steps



A. Post-obturation IOPAR, B. Periodontal probing, C. Incision placed, D. Mucoperiosteal flap raised, E. Resected cystic lining, F. Retro cavity prepared, G. MTA filled retro cavity, H. Prepared sticky bone carried to bony cavity, I. GTR membrane placed, J. Suture placed, K. Immediate post-op, L. 6 month IOPAR

Fig. 8: POST-OP CBCT(6 months follow-up)



IV. Result

From TABLE 1, it appears that bone density of the defect has increased to 57.2% and the gradual reduction in surface area is 75.3% in 6 month follow-up visit in case 1. And also, in case 2 it was observed that bone density of the defect has increased to 51.28% and the gradual reduction in surface area is 81.9% in 6 month follow-up visit.

V. Discussion

After surgical endodontics comprising of apicoectomy and enucleation of large periapical lesion, early bone regeneration is required to provide functional support to the tooth.

The four critical factors which influences the regeneration of bone after periapical surgery are (PASS principles)[8]:

1. primary wound closure,
2. angiogenesis to provide blood supply to surgical wound & source of undifferentiated mesenchymal cells,
3. space maintenance, and
4. stability of the blood clot.

Sticky bone, formed from AFG (platelet concentrate) mixed with particulate HA crystals improves the handling properties of graft material by providing stability, wound closure, haemostasis, and maintenance of space of bony crypt to be replaced by bone tissue.[9]

Clinical trial suggest that the combination of bone graft along with the growth factors in the PRF may be suitable to enhance the bone density.[5] PRF is a rich source of various platelet derived growth factor(PDGF),transforming growth factor β (TGF-β), insulin growth factor(IGF), vascular endothelial growth factor(VEGF), Epidermal growth factor(EGF), etc with their following roles:

1. PDGF – promotes angiogenesis, activates macrophages to initiate the release of growth factors to enhance bone, cementum, and periodontal ligaments reapair and regeneration.[10,11]
2. 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.[12]
3. IGF-I- stimulates bone formation by proliferation and differentiation[13], and it is synthesized and secreted by osteoblasts.[14]
4. VEGF also promotes angiogenesis, increases vascular permeability, stimulates mitogenesis for endothelial cells.[15]
5. EGF stimulates endothelial chemotaxis/angiogenesis, regulates collagenase secretion and epithelial msenchymal mitogenesis.[15]

An increase in the proliferation of human osteoblasts have been demonstrated with a combination of PDGF,TGF-β, IGF, VEGF, EGF.[16]

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.[17](2011) 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)[18] and then invade the underlying connective tissue. 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 [19,20] tissue is formed in large bone

defects. Barrier membranes are thought to prevent movement of proliferating fibroblasts from the surrounding tissue [21,19,20] into the bony defect. 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 [22] movement. Importantly, during periapical bone wound healing after apical surgery, the newly differentiated osteoblasts are derived from bone marrow mesenchymal stem cells and osteoprogenitor [23] cells lining the endosteum. 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[24] 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 the incisors on left side with the dehiscence (apicomarginal) defect on the labial surface of the lateral incisor with discharging pus through the deep periodontal pocket in both the cases. In the management of this case, Sticky bone (HA and AFG mixture), in combination with PRF membrane and GTR membrane were used. Satisfactory result was observed after follow-up period of 6 months only.

VI. Conclusion

Regeneration of bone in bony crypt after apicoectomy and bony defects like dehiscence can be achieved by using sticky bone in combination with PRF membrane and GTR membrane as barrier membrane.

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