CLINICAL AND HISTOLOGICAL EVALUATION OF PERIODONTAL REGENERATION IN VERTICAL OSSEOUS DEFECTS TREATED WITH PLATELET – RICH PLASMA

Dr. AVANEENDRA TALWAR
POST GRADUATE STUDENT
PERIODONTIA

INTRODUCTION

- A major goal of periodontal therapy is regeneration of the attachment structures such as alveolar bone, periodontal ligament and cementum. Open flap debridement results in the formation of long junctional epithelium, which is more susceptible to microbial invasion and is thought to be a less stable attachment.
- Regeneration is thought to partially mimic developmental mechanisms, which require a coordinated orchestration of cellular events such as proliferation, migration and differentiation.

- Polypeptide growth factors are naturally occurring biological modifiers that have the potential to alter the host tissue to stimulate or regulate the wound healing process. They can regulate key cellular events in tissue regeneration, including cell proliferation, chemotaxis, differentiation, and matrix synthesis via binding to specific cell surface receptors.
- Growth factors (GF) either singly or in combination have been used and experimental evidence for bone regeneration has been documented in both animal and human trials.
- Platelets are a rich source of naturally occurring growth factors, which can play an important role in regeneration of periodontal tissue.

- **Platelet-Rich Plasma (PRP)** is procured from whole blood and is rich in platelets and naturally occurring autologous growth factors that are present in plasma.
- These growth factors (GFs) are concentrated to about 300 times that of the levels normally present in plasma.
- Although plasma is considered to have more than thirty different growth factors, growth factors such as Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β) present in platelets are the most important, assist in tissue healing, mainly by stimulating proliferation and differentiation of mesenchymal cells.
- The platelet gel by itself does not have adequate space making ability and has to be used along with a carrier such as bone grafts.

- Bone regenerative grafts have been used in the last few decades to enhance bone formation and regeneration of periodontal tissues in the treatment of vertical osseous defects.
- These bone grafts help in bone formation by processes such as Osteoconduction, Osteoinduction, or Osteogenesis.
- **Hydroxylapatite (HA),** which is an inert inorganic synthetic alloplastic graft material has been used extensively in the treatment of osseous defects.
- The porous form of HA is formed through a hydrothermal conversion of the calcium carbonate exoskeleton of coral.
- The HA formed can support fibrovascular ingrowth and subsequent bone formation, i.e., it helps in bone formation by the process of osteoconduction.

- The lack of osteoinductive potential is a limit to the use of **Porous Hydroxylapatite (PHA)** in the treatment of intrabony defects.
- Addition of PRP to synthetic porous hydroxylapatite can assist in jump-starting the cascade of events that might lead to the formation of new bone, by delivery of growth factors to the healing site.
- These growth factors can begin and maintain the differentiation and proliferation of osteoblastic/ progenitor cells into the space occupied by the osteoconductive HA.
- The present study was carried out to evaluate improvement in clinical parameters and gain histologic evidence for the formation of new attachments in periodontal lesions in patients treated with a combination of PRP and synthetic porous HA (OsteoGen).

AIMS AND OBJECTIVES

- The aims of this study were: -

1. To evaluate the improvement in clinical parameters in patients treated with PRP/porous synthetic hydroxyapatite combination with respect to use of synthetic porous hydroxylapatite alone.
2. To histologically evaluate the efficacy of Platelet-Rich Plasma in the formation of new attachment at the periodontal lesion treated with Platelet-Rich Plasma and synthetic porous hydroxylapatite (Osteogen).

MATERIALS AND METHODS
Clinical Study

Patient selection

- Twenty systemically healthy patients attending the Out-Patient Clinic of the Department of Periodontics, Ragas Dental College and Hospital, were included in the study. Patients ranging between the age groups 18-60 were chosen. Prior to the execution of the treatment, informed consent was obtained in a written format and the treatment procedure explained to the patient. There were ten patients each in the experimental group and the control group.

Criteria for selection of patients

Inclusion criteria

1. Good health
2. Patients with no known medical problems that would contraindicate routine periodontal therapy.
3. Patients who have not undergone any sort of periodontal therapy for the past six months.
4. Patients diagnosed as having chronic periodontitis.
5. Patients with radiographically detectable vertical osseous and furcation defects.
6. Probing depth > 5mm.
7. Not taking drugs known to affect the periodontal status.
8. Not pregnant or lactating.
9. Patients who were non-smokers.

Exclusion criteria

1. Patients with uncontrolled systemic illness, compromised individuals, pregnant and / or lactating women, and patients taking any drug known to cause gingival enlargement.
2. Patients not compliant and unable to maintain recall visits.
3. Smokers

Pre-surgical clinical measurement

- Prior to surgery, full mouth plaque scores were measured. A Williams periodontal probe was inserted into the pocket at the angle necessary to reach the deepest portion of the interproximal or furcation pocket.
- The probing pocket depth (PPD) and the relative clinical attachment (using the CEJ as reference) were recorded.
- The measurements were repeated on the buccal and lingual surfaces of each defect, if the defect was present on both sides.

- The deepest probing pocket depths were recorded. Standardized radiographs were taken at baseline (before the surgery).
- The measurement of bone loss on the radiograph were done with the help of the formula:

$$\frac{\text{CEJ} - \text{Bone base}}{\text{CEJ} - \text{Root apex}} \times 100 = \%\text{Bone loss}$$

Pre-surgical therapy

- Preoperative hematological assessment included a complete blood count.
- Initial therapy consisted of oral hygiene instructions which were repeated until the patient achieved an Oral Hygiene Index-Simplified score of less than 1 and there was absence of clinical signs of gingival inflammation.
- Scaling and root planing of the quadrant involving the teeth to be treated was performed under local anesthesia. Symptoms of trauma of occlusion if detected were corrected.

- Three weeks following Phase-1 therapy, periodontal re-evaluation was performed based on plaque scores and the presence or absence of the signs of gingival inflammation.
- Chlorhexidine gluconate 0.12% (b.i.d) as mouth wash was advised two week prior to the surgical procedure.
- Platelet-rich plasma was extracted 30 minutes prior to surgery using venipuncture.

Platelet-rich plasma preparation

- 20 ml of blood were drawn from each patient by venipuncture of the antecubital vein in the forearm into a 20ml syringe.
- 10ml of blood was collected into two glass tubes containing 10% trisodium citrate solution as an anticoagulant.
- The glass tubes containing blood were centrifuged at 1200 rpm for 20 minutes, resulting in separation of two fractions; plasma at the top and red blood cells at the bottom.

- The plasma along with the top 2ml of red blood cell was aspirated with the help of “Eppendorff pipettes”.
- This fraction was again centrifuged at 2000 rpm for 15 minutes to get three basic fractions; platelet-poor plasma (PPP) at the top of the preparation (supernatant), PRP in the middle and red blood cell fraction at the bottom.
- The top 80% corresponding to PPP was aspirated with a pipette, leaving the residual (0.5 -2 ml) platelet concentrate.

Surgical procedure and intrasurgical measurements

- Surgical sites were disinfected with chlorhexidine mouthwash prior to the administration of the local anesthesia.
- The surgical procedure was performed by local infiltration of 2% lidocaine containing adrenaline at a concentration of 1:100,000. Buccal and lingual sulcular incisions were used and a mucoperiosteal flap was elevated. Care was taken to preserve as much interproximal soft tissue as possible.
- Complete debridement of the defects as well as scaling and root planing were achieved with the use of an ultrasonic device and hand curettes.
- Intrabony defects after soft tissue debridement were measured from the base and the crest of the defect to the CEJ.

Treatment of the defects was as follows:

- In the experimental group, at the time of the application of the bone graft, the synthetic, osteoconductive, non-ceramic form of hydroxylapatite (Osteogen - Impladent, New York) was mixed with the PRP preparation in a proportion of 1:1.
- Coagulation of PRP/synthetic HA mixture was achieved by its combination with 5 μml of 10% calcium chloride. Within a few seconds, it assumed a sticky gel consistency.
- The synthetic HA/coagulated PRP mixture was then tightly packed into the bony defects using a plastic condenser to the level of the bony crest. In the control group only synthetic hydroxylapatite was used to fill the defect.

- Flaps were sutured at the original level with black braided silk (4-0) using interrupted sutures.
- Antibiotics (Amoxicillin 250 mg every 6 hours for 5 days) and 0.12% chlorhexidine gluconate rinse (every 12 hours for two weeks) were prescribed.
- Oral analgesic (Ibuprofen 400 mg every 8 hours as necessary) was also prescribed.

Post-operative care

- The periodontal dressing and sutures were removed two weeks postoperatively.
- Surgical wounds were gently cleansed with 0.12% chlorhexidine gluconate on a cotton swab. Patients were instructed to rinse during the second postoperative week.
- Mechanical oral hygiene consisting of brushing was initiated by the patient at the end of the second post operative week.
- Patients were examined weekly up to one month after surgery and then at three and nine months. Postoperative care included reinforcement of oral hygiene and mechanical plaque removal, wherever necessary. Standardized radiographs were taken at 3 months and 9 months post-operative.

Study Design

- Among the patients visiting the out patient department of Periodontics, a general examination of the oral cavity was performed along with a through medical history. Patients who satisfied the above-mentioned inclusion and exclusion criteria were selected.
- A total of 20 patients were chosen for the clinical study. 10 patients were included in each group, namely, the experimental group and the control group.
- All patients were thoroughly educated in oral hygiene routines before the start of the study and maintained at a high level of oral hygiene through out the experimental period.
- The patients in the experimental group received PRP/synthetic hydroxylapatite, while patient in control group received only synthetic hydroxylapatite.

- The following clinical and radiological parameters were recorded at baseline and at 3 and 9 months after the surgery.

1. Plaque Index (Silness and Loe,1964) [129]
2. Gingival index (Loe and Silness, 1963) [74]
3. Probing pocket depth
4. Relative clinical attachment
5. % bone/defect fill

Plaque Index (PI) –Silness and Loe, 1964

- Plaque scoring was done on mesial, facial, distofacial and lingual areas of the tooth selected after drying the teeth, as follows:

Criteria

0 - No plaque

1 - A film of plaque adhering to the free gingival margin and adjacent areas of the tooth. The plaque may be recognized only after application of disclosing agent or by running the explorer across the tooth surface.

2 - Moderate accumulation of the soft deposits within the gingival pocket that can be seen with the naked eye or on the tooth and gingival margin.

3 - Abundance of soft matter within the gingival pocket and/or the tooth and gingival margin.

$$\text{Plaque score} = \frac{\text{Sum of scores for all surfaces}}{\text{No. of surfaces examined}}$$

Rating

- Excellent - 0
- Good - 0.7 -1.7
- Fair - 1.8 - 3.4
- Poor - 3.5 - 5.0

Gingival Index (GI) – Loe and Silness, 1963

- The tissue examined were divided into 4 units, mesiofacial and distofacial papilla, facial margin and lingual margin.

Criteria

- 0 - Normal gingival
- 1 - Mild inflammation: Slight edema, slight change in colour. No bleeding.
- 2 - Moderate inflammation : Redness, edema, glazing. Bleeding on probing.
- 3 - Severe inflammation : Marked redness and edema. Ulceration. Tendency to spontaneously bleed.

Rating

- Excellent - 0
- Good - 0.1- 0.9
- Fair - 1.0-1.9
- Poor - 2.0-3.0

Probing pocket depth (PPD)

- Probing pocket depths were recorded using William's graduated periodontal probe. Probing depths were recorded with, the probe parallel to the long axis of the selected tooth, from the gingival margin to the base of the pocket. The deepest PPD were recorded for each site. The subsequent measurements were also taken at the same location.

Relative attachment level

- Clinical attachments were recorded using Williams graduated periodontal probe. The probe was placed parallel to the long axis of the tooth and the reading recorded.

Radiographic assessments of percentage bone fill

- Intra-oral periapical radiographs of each defect site was obtained using long cone/ paralleling radiographic technique. These radiographs were exposed using a standardized tube voltage and cone–film distance. A grid was incorporated into the films with 1 mm markings to facilitate easy measurement. The measurements on the radiograph were done with the help of the formula:

$$\frac{\text{CEJ – Bone base}}{\text{CEJ – Root apex}} \times 100 = \%\text{Bone loss}$$

STATISTICAL ANALYSIS

- Data was expressed as mean ± standard deviation of the parameters evaluated. In the control and experimental group, the parameters were evaluated at baseline, 3rd month and 9th month. Comparisons were made within each group between the baseline, 3rd month and 9th month evaluation using the one-way analysis of variance (ANOVA). Intergroup comparisons were done at baseline, 3rd month and 9th month using the unpaired t-test. Significance was established at $p < 0.05$.

Histological Study

Patient Selection

- Patients who had two or more maxillary or mandibular incisors, canines, premolars recommended for extraction by the Oral Diagnosis Department and teeth with Grade III to IV furcation involvement which are indicated for radisection were solicited. Patients were systemically healthy. Teeth chosen for the study demonstrated advanced bone loss, deep pockets, and associated vertical osseous defects detected radiographically. Patients were instructed both orally and in writing as to the nature of the study to obtain their informed consent.

Inclusion Criteria

1. Vital teeth with advanced periodontitis and gingival recession indicated for radisection were selected
2. Lingually placed teeth with osseous vertical defects

Surgical Therapy

- All surgical procedures and clinical measurements were performed as described above.
- At vertical osseous defects platelet rich plasma in combination with porous synthetic hydroxyapatite were used.
- Postoperative maintenance was performed weekly for the first month, every other week for the second month and monthly until biopsy.

- Biopsy was performed at 1st, 2nd, 3rd and 5th month at different sites and the specimen fixed in 10% neutral formalin.
- The patients were asked to report after 10 days to remove the sutures and to debride the wound.
- After the surgical sites had healed, patients were advised to report for a review at said intervals to check for wound healing and vitality of the teeth. [129]

Processing of the specimen

The specimen were processed as follows:

- Specimen were decalcified in 20% formic acid. The formic acid was changed every hour.
- The specimen were put in 10% formalin overnight. This process was carried out for 20 days or until the specimen were fully decalcified.
- The decalcified sections were washed in running water for half an hour and fixed with 10% formalin for 24 hours.

- After fixation in formalin, the specimens were washed for half an hour in running water.
- They were gradually dehydrated by passing through a series of increasing percentages of alcohol (70%, 90% and absolute alcohol), remaining in each dish for half an hour.
- The specimen were kept in absolute alcohol overnight.
- They were then passed from alcohol through two changes of xylene for half an hour each.

- They were then removed from Xylene and placed in a dish of melted embedding paraffin, and the dish was put into a constant temperature oven regulated to about 60°C.
- The specimen were then changed to two successive dishes of paraffin for half an hour each.
- The specimen were then embedded in the centre of a paraffin block and cut into 5-micron sections using a microtome.

- Suitable lengths of paraffin ribbons were floated in a pan of warm water.
- Slides coated with a thin film of Meyer's albumin adhesive were slipped under the ribbons and then lifted from the water with the ribbons. The slides were allowed to dry.
- The sections were stained using Harris's haematoxylin, 1% eosin and 1% HCl.

- Harris's Haematoxylin solution contains:

- Haematoxylin	2.5 gms
- Absolute alcohol	25 ml
- Distilled water	500 ml
- Glacial acetic acid	20 ml
- Potassium alum	50 gm
- Mercuric oxide	1.25 mg

- In H and E staining, a weak dye called haematein is used in combination with Al^{3+} ions; alum haematoxylin. The dye complex formed, has a deep purple colour.
- The second stain is eosin, which imparts a pink-to-red colour to most tissue components not stained bluish-purple by haematoxylin.

- The sections were stained with haematoxylin for 5 minutes and washed in running tap water for 5 minutes till colour sections were blue.
- This was then differentiated in acid alcohol (1% HCl in 70% Alcohol) for a few seconds, followed by washing in running tap water for 5 -10 minutes.
- Then the sections were counterstained with 1% Aqueous Eosin for 30 seconds. These were again washed in tap water and dehydrated in two changes of Absolute Alcohol for 1-2 minutes, cleared in Xylol and mounted in Canada Balsam.
- Histologic evaluations of the specimen were conducted by an oral pathologist.

RESULTS

Clinical findings

- Two weeks after the grafting procedure, the first clinical examinations were carried out. All the 20 grafted sites demonstrated good healing without signs or symptoms of infection. The clinical parameters such as Plaque index, Gingival index, probing pocket depth and relative attachment loss were recorded at baseline (prior to therapy) and were carried out at the 3^{rd} and 9^{th} months post surgically.

Probing pocket depth (PPD)

- The baseline mean probing pocket depth for experimental group was 6.8 ± 2.2. At the 3rd month, it was 2.8 ± 0.79 and at the 9th month, it was 3.3 ± 1.42. The changes in probing pocket depth were statistically significant ($p < 0.001$).
- The baseline mean probing pocket depth for control group was 8.1 ± 1.85. At the 3rd month, it was 4.1 ± 0.57 and at the 9th month, it was 3.6 ± 0.84. The change in probing pocket depth was statistically significant ($p < 0.001$). .
- Between the groups, the reduction in probing pocket depths was statistically significant ($p < 0.001$) at the 3rd month.

Relative attachment level (RAL)

- The baseline mean relative attachment level for experimental group was 7.5 ± 2.42. At the 3rd month, it was 3.9 ± 1.792 and at the 9th month, it was 4.4 ± 2.17. The relative attachment level gain was statistically significant ($p < 0.016$).
- The baseline relative attachment level for control group was 7.5±1.96. At the 3rd month, it was 4.9 ± 0.74 and at the 9th month, it was 4.86 ± 1.14. The relative attachment level gain was statistically significant ($p < 0.002$). .
- Between the groups, the relative attachment gain was statistically insignificant.

Plaque Index (PI)

- The baseline mean plaque index scores for experimental group was 1.2 ± 0.63. At the 3rd month, it was 0.35 ± 0.39 and at the 9th month, it was 0.48 ± 0.40. The change in plaque index scores was statistically significant ($p < 0.01$).
- The baseline mean plaque index for control group was 1.1 ± 0.57. At the 3rd month, it was 0.28 ± 0.28 and at the 9th month, it was 0.35 ± 0.24. The change in plaque score index was statistically significant ($p < 0.01$). .
- Between the groups, the change in plaque score at baseline, 3 months and 9 months was statistically insignificant

Gingival Index (GI)

- The baseline mean gingival index scores for experimental group were 1.8± 0.42. At the 3rd month, it was 0.33 ± 0.33 and at the 9th month, it was 0.78 ± 0.67. The change in gingival index scores was statistically significant ($p < 0.000$).
- The baseline mean gingival index for control group was 1.7 ± 0.48. At the 3rd month, it was 0.18 ± 0.24 and at the 9th month, it was 0.40 ± 0.58. The change in gingival score index was statistically significant ($p < 0.000$). .
- Between the groups, the change in gingival score at baseline, 3 months and 9 months was statistically insignificant.

Radiographic findings

- The baseline mean percentage bone loss in the experimental group was 24.87 ± 13.71. At the 3rd month, the percentage bone loss reduced to 19.33 ± 11.64 and at 9th months it was 19.83 ± 11.50.
- The percentage bone loss was statistically insignificant ($p < 0.55$) at 3 months and 9 months suggestive of good bone defect fill.
- The baseline mean percentage bone loss in the control group was 37.78 ± 163.28. At 3 months, the percentage bone loss was 40.6 ± 13.31 and at 9 months, it was 19.83 ±13.49.

- The percentage bone loss were statistically insignificant ($p < 0.4$) at 3 months and 9 months, suggestive of good bone defect fill.
- The intergroup reduction of percentage bone loss was statistically significant at 3 months ($p < 0.001$) and at 9 months ($p< 0.46$).
- But this was due to the fact that the baseline data were not similar, being higher for the control group with respect to the experimental group.

Histologic findings

- Following surgery the tooth or the most compromised roots were extracted at 1,2,3,4 and 5 months in different patients and the specimens obtained were processed and examined under light microscope.
- The photomicrograph of specimen taken 1 month after grafting showed the presence of sparse inflammatory cell, immature bone formation with mature collagen (Fig. 1).
- The photomicrograph of specimen taken at 3 months after grafting showed a small amount of mineralised bone with fibrous connective tissue.

- The photomicrograph of specimen taken at 4 months showed presence of mature bone (Fig. 2).
- The photomicrograph of specimen taken at 5 months showed mature bone with osteocytes within lacunae along with the presence of resting lines.
- There was also the presence of cellular connective tissue. None of the slides showed periodontal ligament or acellular cementum (Fig.3 & 4).

DISCUSSION

- The limitations of traditional therapies have promoted the development of tissue engineering.
- The emerging field of tissue engineering is concerned with the development of natural biological surrogates that restore, maintain, or improve upon tissue structure and function.
- Three general strategies have emerged for engineering of tissues. The first is a conductive approach, in which synthetic scaffold materials amenable to infiltration of specific cell types are implanted into a site of disease or damage.

- The materials provoke conduction of desired cell types while blocking conduction of unwanted cell types. The second approach involves the inclusion of bioactive factors (e.g. growth factors) into the aforementioned synthetic scaffolds.
- The factors are chosen to spur the infiltration of the specific cell types, and induce the formation of a specific type of tissue.
- The third approach is based on seeding scaffolds with cells in vitro, followed by implantation of the cell construct.

- Basic and clinical research has focused on the application of growth factors for the regeneration of tissues.
- This can be achieved through gene therapy. In a review, **Yao and Eriksson** (2000) reported that short shelf life and inefficient delivery to target cells are the major concerns associated with local administration of recombinant human growth factors.
- The growth factors were expensive and many doses were required to achieve any obvious therapeutic effect.

- Another easy, cost-effective way to obtain concentrations of growth factors for tissue healing and regeneration may be autologous platelet storage via PRP.
- The rationalization of the use of PRP as a bone regenerative stimulating agent lies in the possibility of concentrating the growth factors contained in platelets and carrying them into the regenerating site with an ideal carrier, like the patient's platelets.
- The ability of PRP to enhance the consolidation of bone graft has been well established since 1998 by the pioneering works of **Robert E Marx et al**.

- Several studies have exemplified the role of platelet formulations in regeneration of soft/hard tissues, including formation of new bone.
- In these studies different types of bone replacement materials such as demineralized bone powder, Bio-bone/Bio-Oss, hydroxylapatite and other forms of allografts have been used in combination with PRP gel.
- **Siebrecht et al** (2002) demonstrated increased bone ingrowths into porous hydroxylapatite in a bone chamber rat model when used in combination with platelet concentrate.

- During the bone regeneration process, the growth factors carry out important functions for the initiation and maintenance of the differentiation and proliferation of the osteoblastic precursor cells and osteoblasts themselves, which lead to bone formation.
- During the first stage, the TGF-β and PDGF are released by the platelet clot, stimulating the maturation of osteoblastic precursors towards the mature phenotype in association with the growth factors released by the bone tissue.
- This primary stimulation in tissue healing causes specifically, the expression and synthesis of other regulatory factors from macrophages and osteoblasts attracted to the regenerating site.

- These include the bone morphogenic proteins synthesized by endosteal osteoblasts, which help the continual osteoblastic differentiation of the mesenchymal cells.
- Once started, the regeneration process is able to maintain a high level through the synthesis of the same growth factors by the osteoblast involved in the process, also supported by capillary angiogenesis specifically promoted by PDGF.
- The present study was a prospective, clinical and histological randomized study done to evaluate the improvements in the clinical parameters and to demonstrate new attachment histologically in vertical osseous defects treated with PRP/porous synthetic hydroxylapatite (OsteoGen) combination.

- OsteoGen, (Implant, Holleswood, NY) is a porous synthetic non-ceramic form of hydroxylapatite, whose particulate material is processed at low temperature, which is resorbable, with particles measuring 300 to 400 μm.
- It contains no alpha or beta- tricalcium phosphates or pyrophosphates. It is a highly crystalline material with no amorphous phases.
- This non-sintered hydroxylapatite resorbs acting as a mineral reservoir inducting bone formation via osteoconductive mechanism. Its reported advantage is the slow resorption rate, allowing it to act as a mineral reservoir at the same time acting as a scaffold for bone replacement.
- OsteoGen was used in the study as a carrier for platelet rich plasma.

- Twenty patients who were diagnosed with generalized chronic periodontitis took part in the clinical study.
- They were randomly assigned to either the experimental group or control group, such that each group had ten patients.
- For patients in the experimental group, the osseous defects were treated by flap surgery followed by placement of PRP/porous synthetic hydroxylapatite (OsteoGen) combination into the vertical osseous defects.
- In the control group, the vertical osseous defects received porous synthetic hydroxylapatite (OsteoGen) alone.

Inclusion criteria for selection of patients for the study included

- good health,
- systemically healthy patients,
- patients who had not undergone periodontal therapy for the past six months,
- patients diagnosed as having chronic periodontitis,
- probing pocket depth greater than 5 mm,
- patients with radiographically detectable vertical osseous and furcation defects. **Exclusion criteria** included patients with
- uncontrolled systemic disease,
- patients not compliant and unable to maintain recall visits,
- smokers.

- The procurement of autologous venous blood was done prior to administration of local anesthesia in patients undergoing periodontal flap surgery.
- Platelet rich plasma was procured by the technique proposed by **Sonnleitner and Sullivan** in 2000.
- A similar technique was used by **Robert Zimmerman and Jakubeitz** in 2001 and by **George Weibrich and Willfred Wagner** in 2001 and 2002.
- In this study, the methods used were similar to those used by the authors mentioned above, except that thrombin was not used, so as to avoid any risk to the patient by mixing PRP with any other component of animal or human origin.

- **Landensberg et al** (1998) in a letter to the editor brought to attention, reports of the use of Bovine thrombin resulting in the development of antibodies to factor V, IX and thrombin, resulting in life threatening coagulopathies.
- In our study, we found that addition of platelet gel to the bone graft prior to packing made it more stable and adherent, which facilitated easy packing into the vertical osseous defects.
- In the clinical study, the following parameters were recorded;
  - Probing pocket depth (PPD)
  - Relative attachment level (RAL)
  - Plaque Index (PI)
  - Gingival Index (GI)
  - Percentage defect fill

- The changes between baseline (pre-operative), 3rd month and 9th month in periodontal pocket depth, relative attachment level, plaque index, gingival index and percentage bone gain are presented in a table.
- In this study, the control group showed a mean change in probing pocket depth of 8.1 ± 1.85 to 4.1 ± 0.57, baseline to 3rd month and 8.1 ± 1.85 to 3.6 ± 0.84, baseline to 9th month. The reduction in probing depth was statistically significant ($p < 0.001$) from baseline to 3rd month and 9th month.
- The changes in relative attachment level in the control group was 7.5 ± 1.96 to 4.9 ± 0.74, baseline to 3rd month and 7.5 ± 1.96 to 4.8 ± 1.14, baseline to 9th month. The reduction in relative attachment level was statistically significant ($p < 0.002$).

- The changes in plaque index scores in the control group was 1.1 ± 0.57 to 0.28 ± 0.28, baseline to 3rd month and 1.1 ± 0.57 to 0.35 ± 0.24, baseline to 9th month. The reduction in probing depth was statistically significant ($p < 0.001$).
- The changes in gingival index scores in the control group was 1.7 ± 0.48 to 0.18±0.24, baseline to 3rd month and 1.7 ± 0.48 to 0.35 ± 0.24, baseline to 9th month. The reduction in probing depth was statistically significant ($p < 0.001$).
- The percentage bone loss for control group at baseline was 37.78 ± 16.28% at 3rd month the percentage bone gain was 40.6 ± 13.31% and at 9th month was 31.86 ± 13.49%. The percentage bone gain with respective to percentage bone loss was statistically insignificant ($p<0.4$) suggestive of good defect fill.

- This was in conformation with clinical defect fill, probing depth reduction and attachment gain reported **by Kenney at al** (1985) [57], **Bowen et al** (1989) [13], **Mora et al** (1995) [97] when porous hydroxylapatite alone was used. The reduction in probing depth could be due to resistance offered by the graft material to the periodontal probe.
- The experimental group showed a mean change in probing pocket depth of 6.8 ± 2.2 to 2.8 ± 0.79, baseline to 3rd month and 6.8 ± 2.2 to 3.3 ± 1.42, baseline to 9th month. The reduction in probing depth was statistically significant ($p < 0.001$) from baseline to 3rd month and 9th month.
- The changes in relative attachment level in the experimental group was 7.5 ± 2.42 to 3.9 ± 1.79, baseline to 3rd month and 7.5 ± 2.42 to 4.4 ± 2.17, baseline to 9th month. The reduction in relative attachment level was statistically significant ($p < 0.016$).

- The changes in plaque index scores in the experimental group was 1.2 ± 0.63 to 0.35 ± 0.39, baseline to 3rd month and 1.2 ± 0.63 to 0.48 ± 0.40, baseline to 9th month. The reduction in probing depth was statistically significant ($p < 0.001$).
- The changes in gingival index scores in the experimental group was 1.8 ± 0.42 to 0.33 ± 0.33, baseline to 3rd month and 1.8 ± 0.42 to 0.78 ± 0.67, baseline to 9th month. The reduction in probing depth was statistically significant ($p < 0.011$).
- The percentage bone loss for experimental group at baseline was 24.87 ± 13.71% at 3rd month the percentage bone gain was 19.33 ± 11.64% and at 9th month it was 19.83 ± 11.50%. The percentage bone gain with respective to percentage bone loss was statistically insignificant ($p < 0.55$) suggestive of good defect fill.

- Both treatment procedures showed reduction in probing pocket depth. Between the groups the reduction in probing depth was statistically significant ($p < 0.001$) at third month, with the experimental group showing a reduction of 2.8 ± 0.79 compared to 4.1 ± 0.57 in the control group.
- The gain in relative attachment level in the experimental group was 3.6mm and in control group it was 2.6mm ($p<0.12$) at third month.
- As the baseline values for percentage bone loss were dissimilar, the percentage bone gain at the third month could not be said to be statistically significant.

- In the current study, the site treated with PRP/porous hydroxyapatite presented with improvements in the clinical parameters, similar to those reported by **de Obarrio et al** (2000), **Camargo et al** (2002) [18] and **Lekovic et al** (2002).
- The greater reduction in clinical parameters in the experimental group with respect to the control group at 3rd month could be due to the presence of growth factors, which may jump start the bone formation and at the same time inhibit apical migration of the epithelium.
- Besides, it is known that growth factors at the site of application are effective only for a short duration.
- In the control group, only porous synthetic hydroxylapatite was used and as it induces bone formation by Osteoconduction, which is a slow process, the control group had gain in the long term.

- The results obtained in our study suggest that irrespective of the treatment modality, deeper the defects, more was the clinical attachment gain and defect fill. This was in conformity with studies done by **Gottlow et al**.
- For the histological study, five patients who needed to undergo extraction or radisection due to advanced bone destruction of certain tooth were included.
- These patients, following flap surgery were treated with PRP/porous synthetic hydroxylapatite (OsteoGen) graft combination.
- The tooth or the most compromised root was extracted with a small amount of surrounding soft and hard tissue enblock at 1, 2, 3, 4 and 5 months following surgery and the specimen sent for histological analysis.

- Histological evaluation was performed for four of the five specimens as one specimen was not evaluable due to difficulties encountered during processing.
- The aim of this study was to determine whether the use of PRP with porous hydroxylapatite resulted in enhanced bone regeneration.
- Histologic studies by **Kenny et al** (1986) [58], **Stahl et al** (1987) [134], **Carranza et al** (1987) [20], **Martin et al** (1989) [88] showed that though the use of porous synthetic hydroxylapatite resulted in bone formation, there was limited osteogenesis with the graft particles being encapsulated in the new bone formed.
- Moreover, there was no evidence of cementogenesis and the healing occurred by means of long junctional epithelium.

- In our study, the photomicrograph of a specimen taken at 1 month after grafting showed the presence of sparse inflammatory cells, immature bone formation, but mature collagen.
- There was no evidence of fibrous encapsulation of the graft. The presence of sparse inflammatory cells may be due to the fact that inflammatory exudates may have occurred earlier.
- The type of inflammatory cell could not be identified as markers were not used. Mature bone had not formed, as the period was insufficient.
- The photomicrograph of the specimen taken at 3 months after grafting showed a small amount of mineralised bone with fibrous connective tissue.

- The photomicrograph of specimen taken at 4 months showed presence of mature bone.
- The photomicrograph of specimen taken at 5 months showed mature bone with osteocytes within lacunae along with the presence of resting lines. There was also the presence of cellular connective tissue.
- The histologic results confirmed the formation of new bone. The presence of osteocytes within the bone lacunae along with resting lines is suggestive of lamellar bone.
- The presence of cellular connective tissue at the vicinity of the newly formed bone is suggestive of presence of fibroblasts at the site.
- The absence of encapsulated bone graft is suggestive of rapid resorption of the graft, possibly due to the early inflammatory exudates that occurred at the site of grafting.

- These results demonstrate the osteoconductive properties of porous synthetic hydroxyapatite and the effective amplification of the bone regeneration and maturation processes evident at 3 months after the grafting procedure.
- The resting lines indicate a osteogenic aspect along with osteoclastic resorption. None of the slides demonstrated new periodontal ligament formation or acellular cementum.
- This result was not in conformity with the results reported by **Nevins et al** (2003) [103] who demonstrated periodontal regeneration including bone periodontal ligament and cementum.

- **Kubota et al** (2002)[64] in an in-vivo study showed that osteoclasts in a conditioned medium secreted PDGF–BB homodimer which acted as an osteoblastogensis inhibitory factor. This finding cannot be applied to in-vitro as various other factors may have to be considered.
- Moreover, the growth factors released from the platelets have a short life, thus reducing the negative effect of PDGF-BB.
- It is possible to speculate that the fibrin net, derived from the polymerization of plasmatic fibrinogen present within PRP, provides porous synthetic hydroxylapatite with a scaffold for osteoblastic migration in contact with the surface of the biomaterial, and that platelet growth factors enhanced the regenerative potential.

- Platelet rich plasma, which abounds in PDGF, binds to endothelial cells to initiate capillary ingrowth and TGF-β binds to osteoblasts and stem cells to initiate mitosis and stimulate osteoid production.
- During bone grafting procedures, platelets become entrapped in a graft clot and degranulate within hours mainly releasing PDGF & TGF-β.
- The fibrin network established in the graft is thought to assist the Osteoconduction component of bone regeneration. Thus, the bone formed is more mature and dense.

- These growth factors function to assist the body in repairing itself by stimulating stem cells to regenerate new tissue.
- More release of growths factors into the wound, results in more stem cells being stimulated to produce new host tissue.
- These peptides act both locally and systemically in a self-regulating feedback loop system. PRP “jump starts” the cascade of regenerative events leading to form a mature graft site.
- These growth factors are autologous, nontoxic, non-immunogenic and enhance and accelerate the normal bone regeneration pathways.

- Adherence to the root surface impedes apical migration of epithelial cells and connective tissue cells from the flap.
- Moreover; the adhesive nature of the platelet gel enables more predictable flap adaptation and homeostasis.
- Activation of the platelets within the gel and resultant release of growth factors enhances the wound healing potential of the bone graft.
- PRP gel allows for manipulation of the flap without displacing the bone graft.

- The limitations of this study were that
  - It had a relatively small sample size.
  - It was a short term study.
  - The growth factors released from the platelets were not quantified.
  - The improvement in clinical parameters could not be individually related to either the graft used or the PRP.
- Currently, PRP is neither completely understood nor fully utilized. It is necessary to identify all the remaining growth factors in the platelets and to explore the interaction of these growth factors with one another and with their target cells.
- Further biological as well as histomorphometric investigations are needed to confirm the real value of the combination.

SUMMARY AND CONCLUSION

- A major goal of periodontal therapy is regeneration, defined as “reproduction or reconstitution of a lost or injured part such as alveolar bone, periodontal ligament and cementum to their original levels before they were damaged by the disease process.”

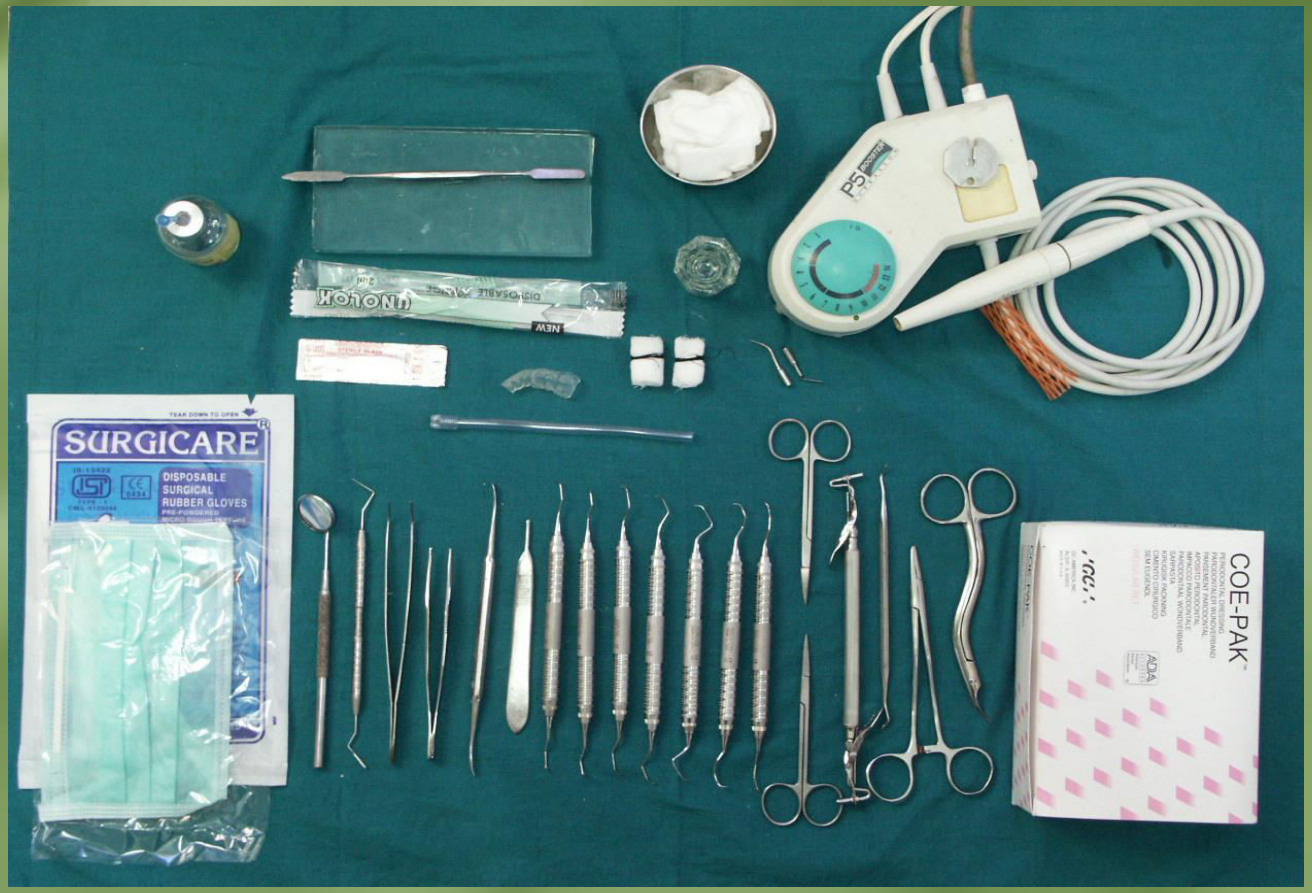
- Although a number of treatment modalities are currently available, clinicians continue to seek more predictable regenerative therapies that are: -
    - Less technique sensitive
    - Lead to faster tissue regeneration
    - Applicable to the broad array of periodontal and peri-implant defects encountered daily by clinicians
- During the last decade, several studies have used biologic mediators such as polypeptide growth factors (GFs) to obtain periodontal regeneration.
- Growth factors are a class of natural biologic mediators that regulate cellular events in tissue regeneration, including cell proliferation, chemotaxis, differentiation and matrix synthesis via binding to specific surface receptors.

- A single growth factor may not be sufficient to help in regeneration.
- Thus, we can use platelet rich plasma which is rich in platelet derived growth factor & transforming growth factor (PDGF & TGF).
- PRP enriched with growth factors is a tool used by surgeons to help improve surgical outcomes.
- These concentrated platelets contain huge reservoirs of growth and wound healing factors, which are natural components of our body.
- These factors enhance and accelerate the body's normal healing process.

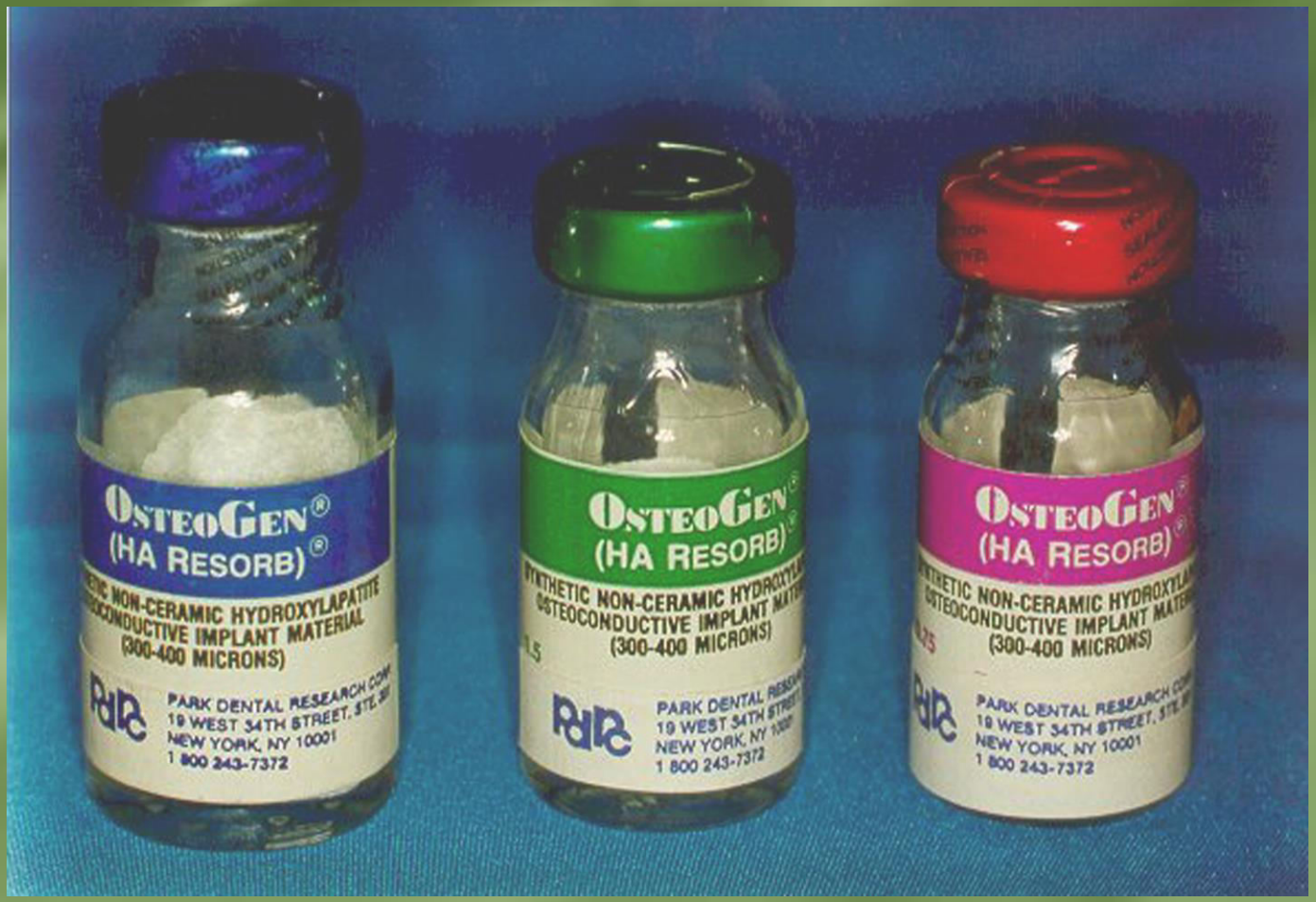
- The clinical and histologic results obtained in this study confirm, in a preliminary manner, the effectiveness of combination of PRP and porous synthetic hydroxylapatite as a grafting material for periodontal regeneration.
- The amplification of growth factors contained within PRP allows for the application of tissue engineering principles.
- No negative effects were found in the use of PRP.
- There was significant reduction in probing pocket depth at 3 months following treatment with PRP. Moreover, the histologic examination showed osseous regeneration of mature bone with complete resorption of the graft material.

- The incorporation of this technique can definitely bring patients benefits, without the risk of infection or disease transmission.
- Future research will essentially confirm and stabilize the best concentration of platelets within PRP for the bone regeneration process and PRP's best use in combination with different grafting materials in different clinical situations.

ARMAMENTARIUM



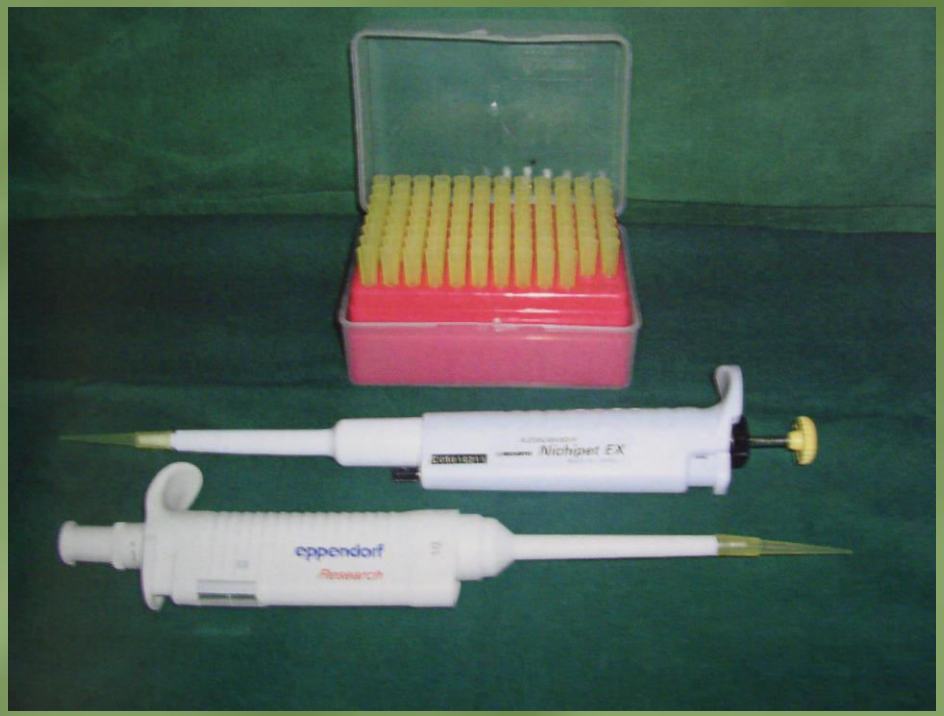
MATERIALS AND INSTRUMENTS USED FOR PERIODONTAL FLAP SURGERY



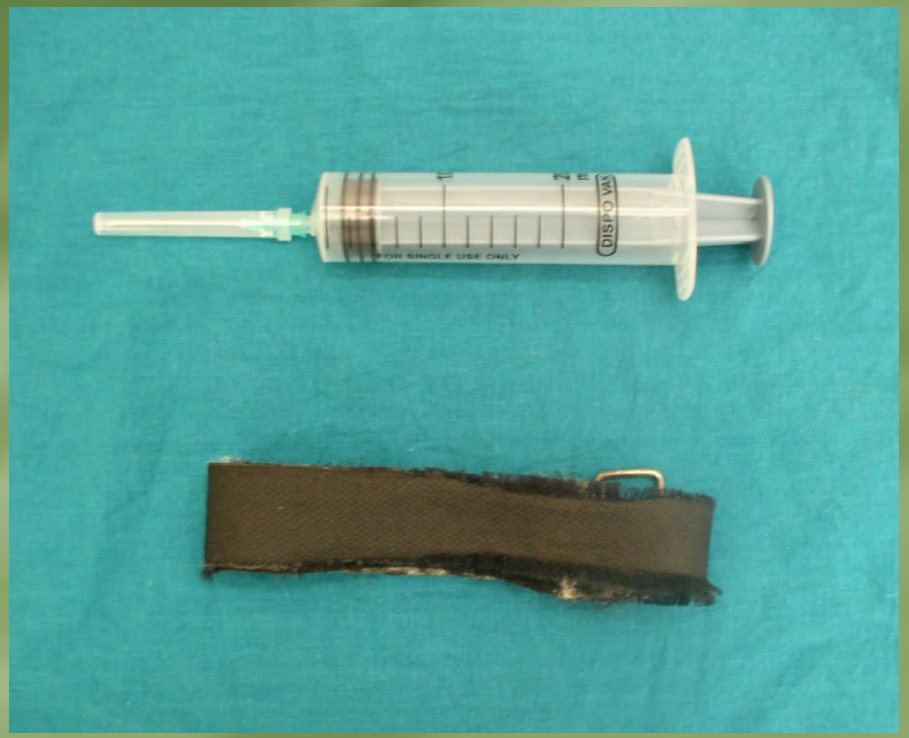
OSTEOGEN BONE GRAFTING MATERIAL

MATERIALS FOR PRP PREPARATION

DUAL BLOOD BAGS
WITH ANTI COAGULANT

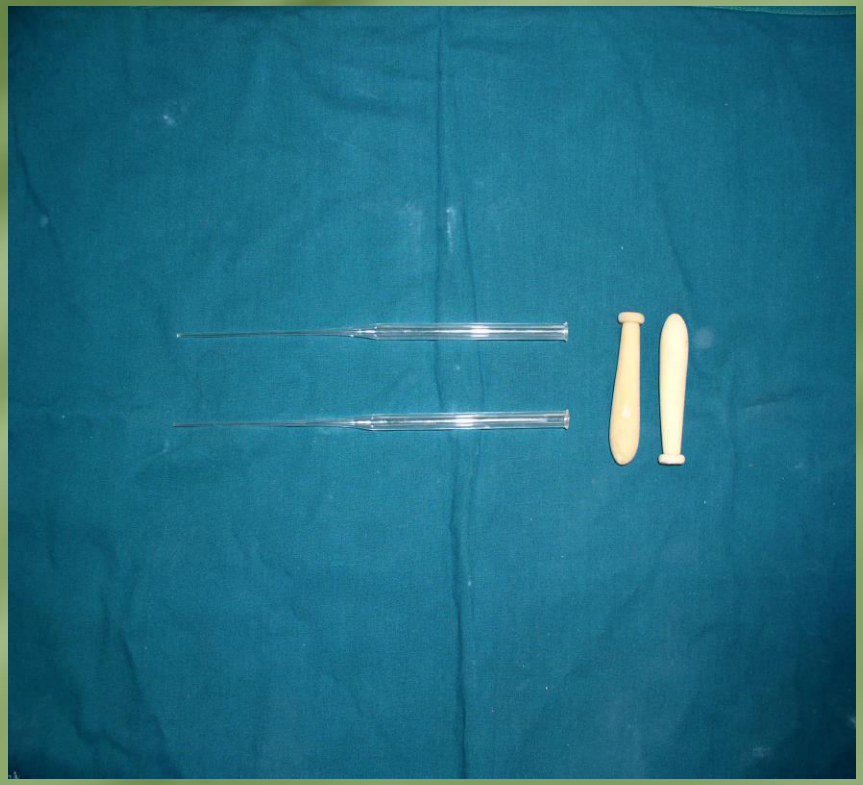


EPPENDORF MICRO PIPETTES
WITH STERILE TIPS



20 ml SYRINGE & TORNIQUET

10% CALCIUM CHLORIDE



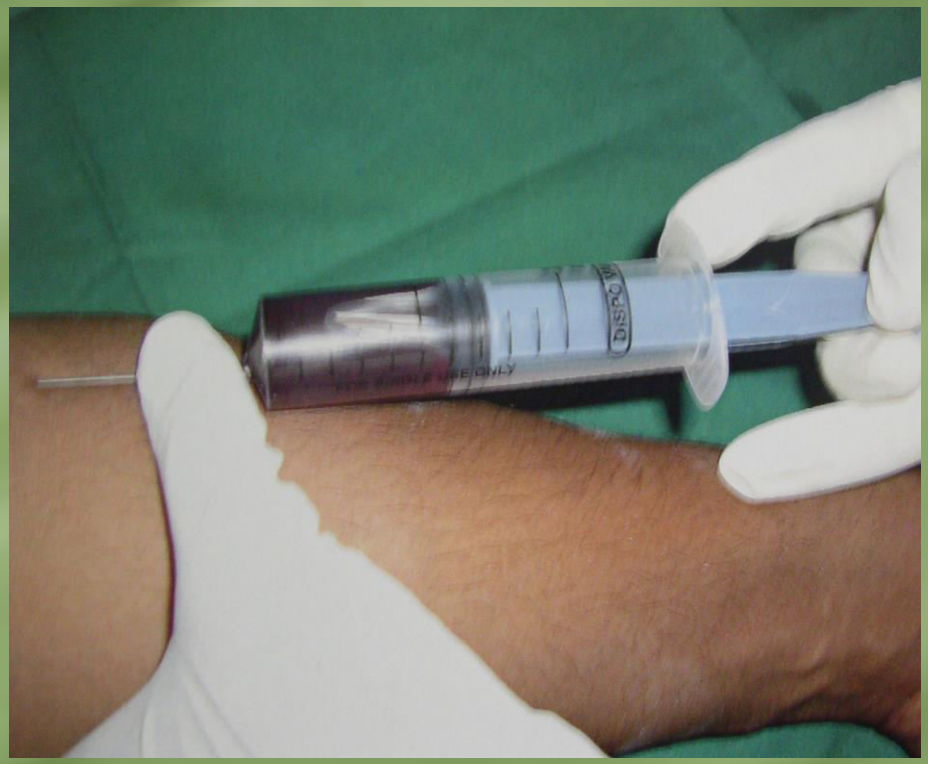
STERILE PIPETTES

AUTOCLAVED TEST TUBES WITH ANTI COAGULANT

UV WORKING CHAMBER

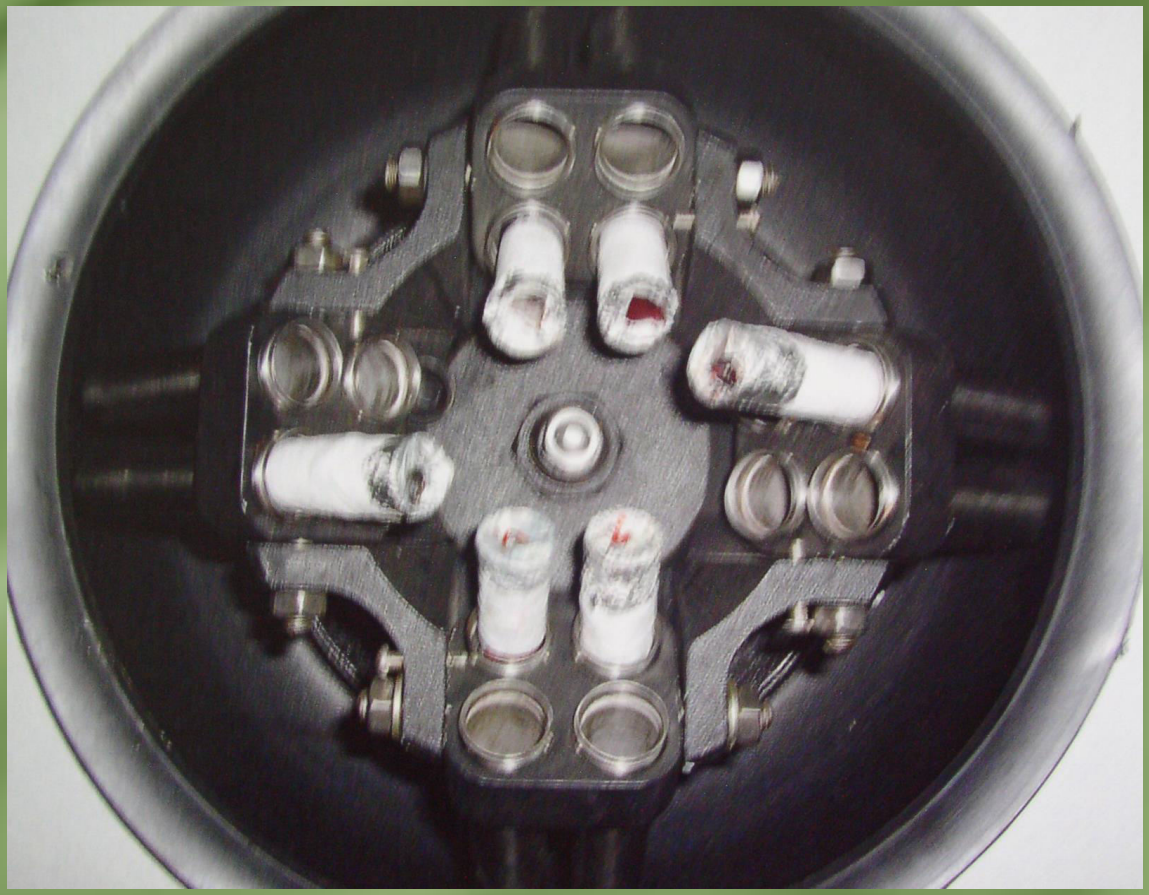
HOT AIR OVEN

METHOD OF PRP PREPARATION

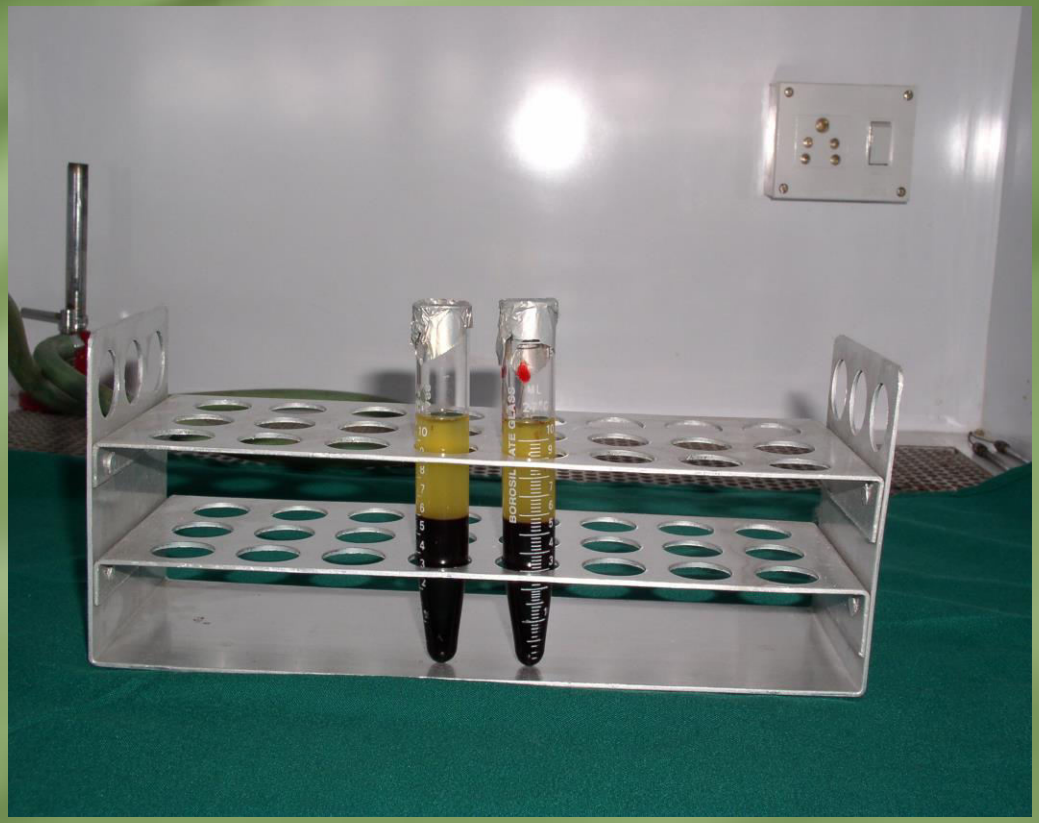


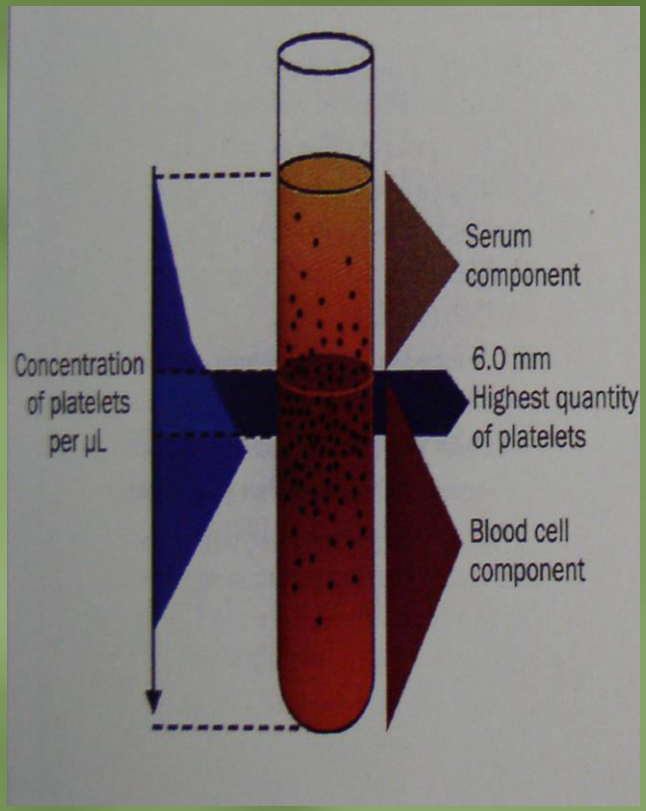
VENIPUNCTURE

BOROSIL TEST TUBES CONTAINING ANTICOAGULANT & BLOOD



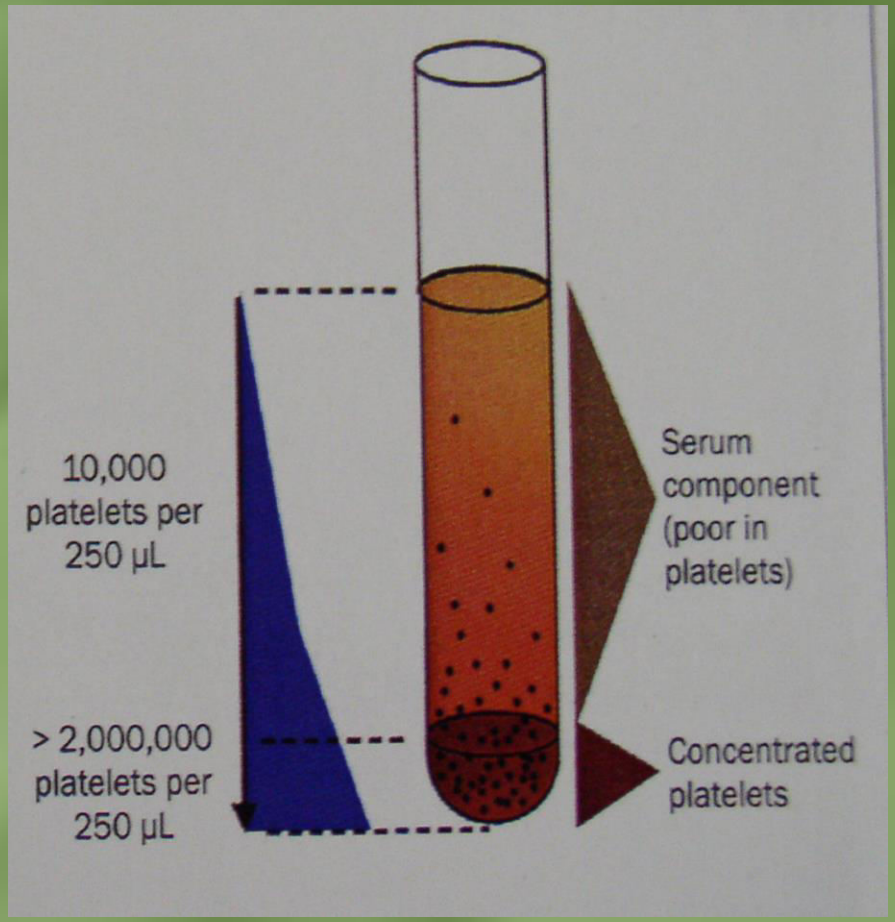
CENTRIFUGING THE BLOOD



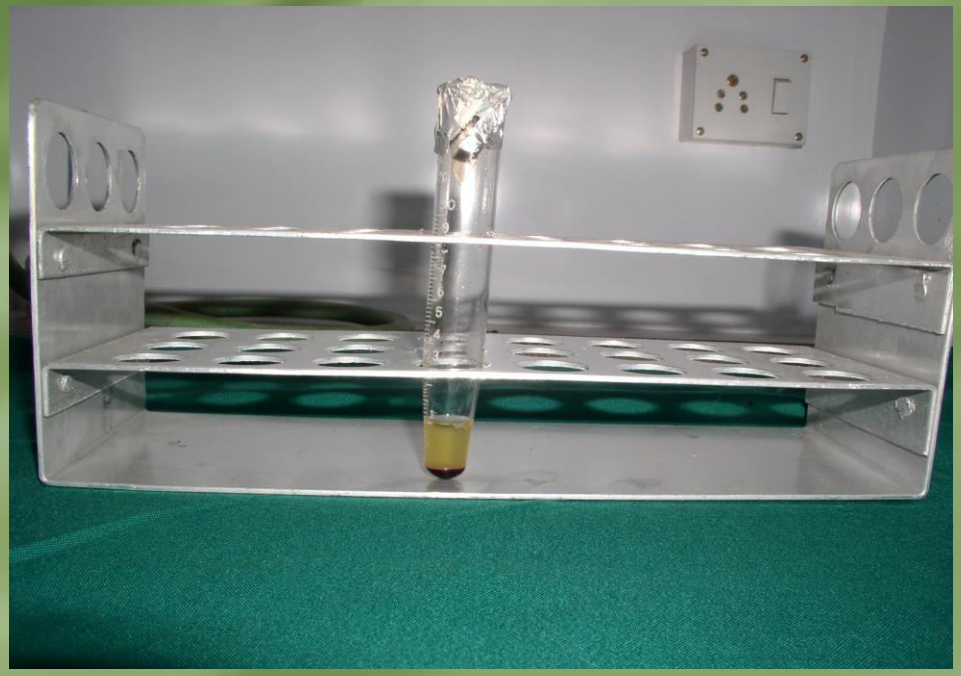


FIRST CENTRIFUGE

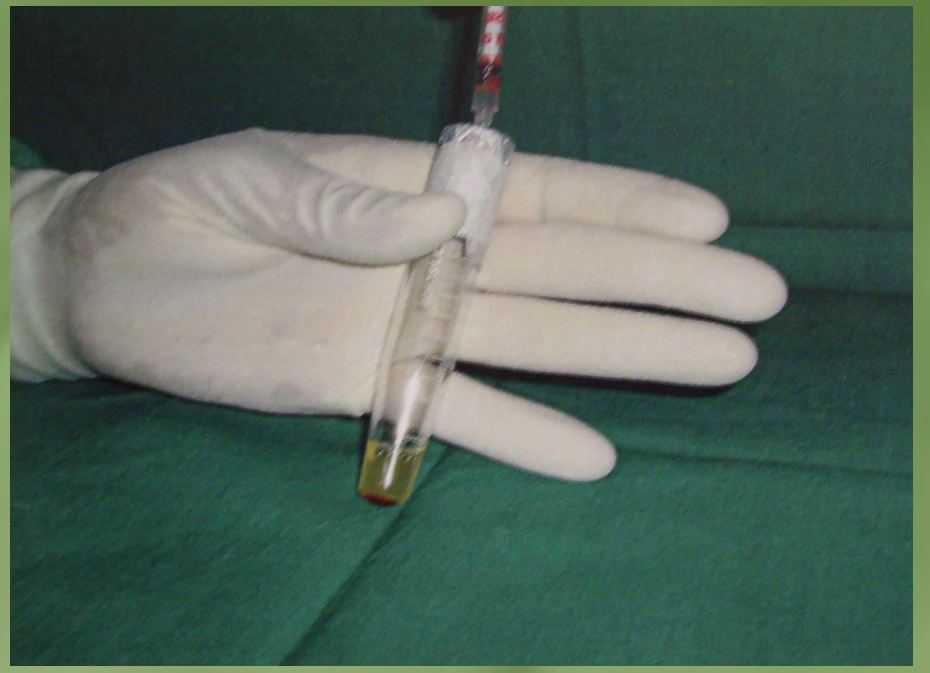
PPP AND PRP SEPARATED WITH 1 ml OF RBC CELLS



SECOND CENTRIFUGE



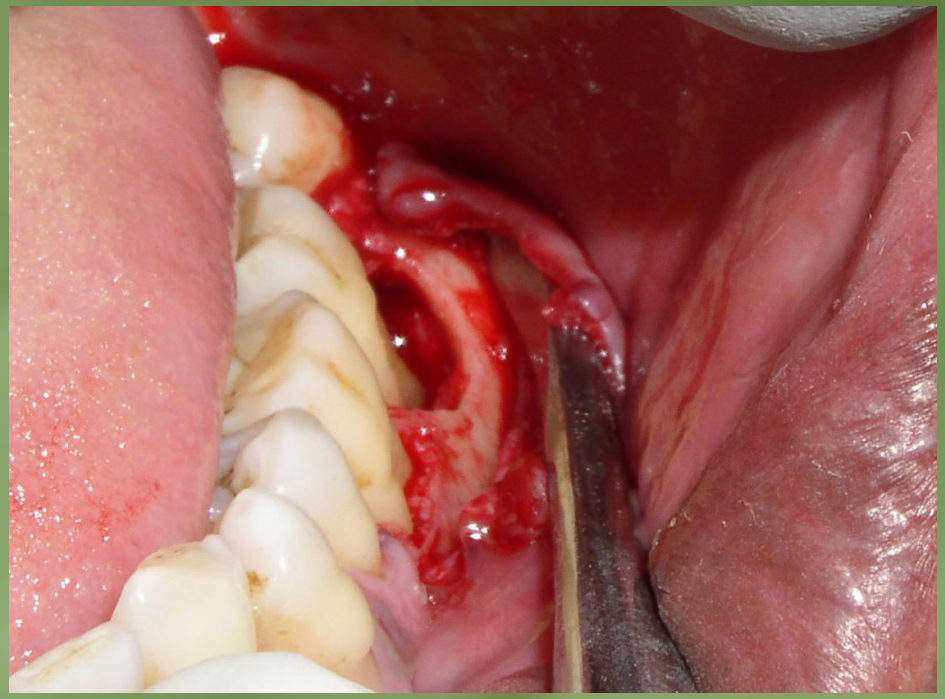
PLATELET BUTTON SUSPENDED IN PRP



10% CALCIUM CHLORIDE ADDED TO PRP

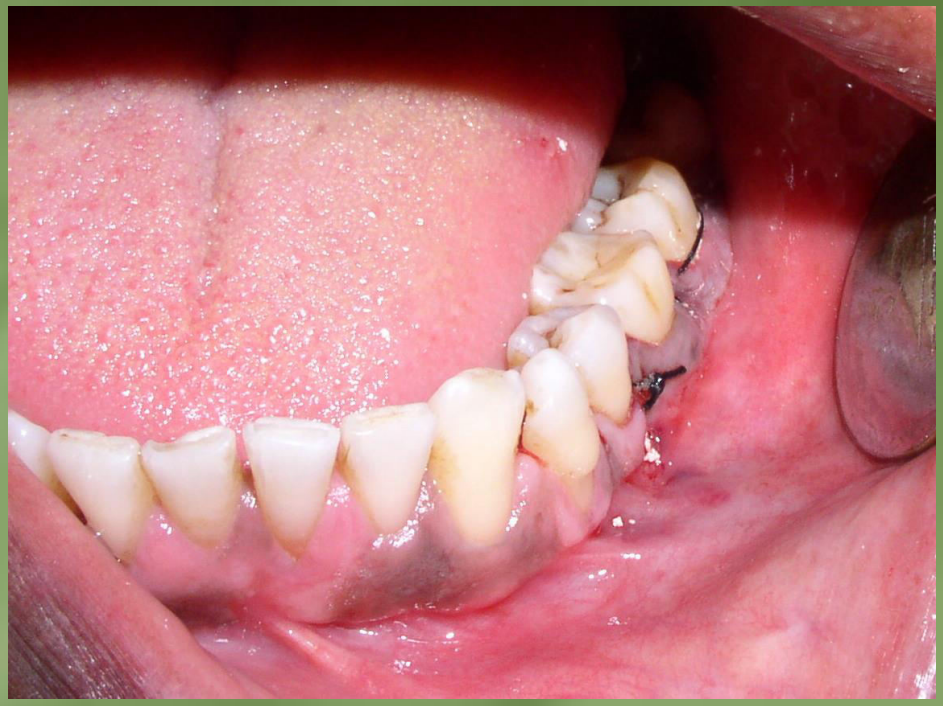
SURGICAL PROCEDURE – CONTROL SITE

PRE OPERATIVE

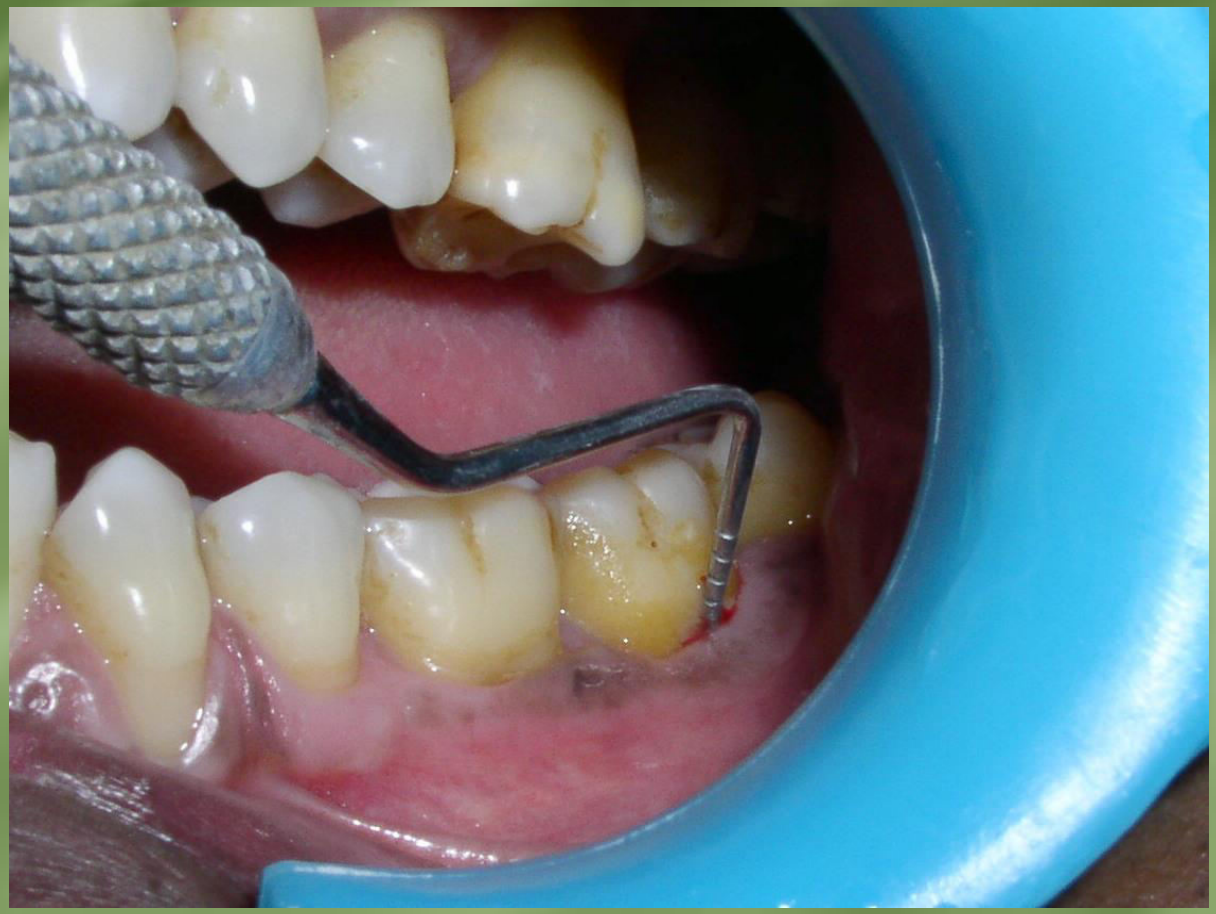


FLAP RAISED & ROOT PLANING, SOFT TISSUE DEBRIDEMENT DONE

POROUS HYDROXYLAPATITE GRAFT PLACED IN DEFECT

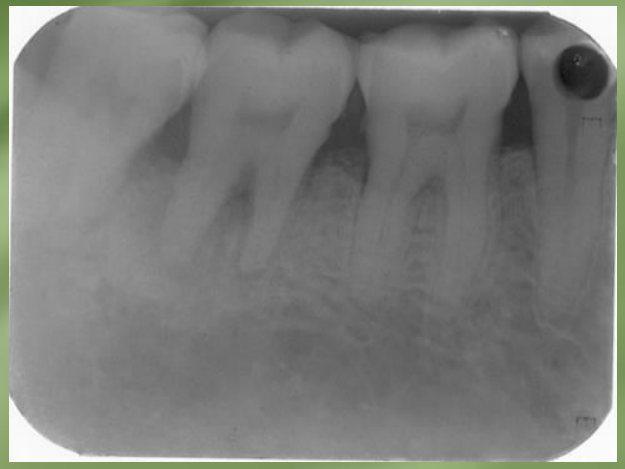


SUTURES PLACED

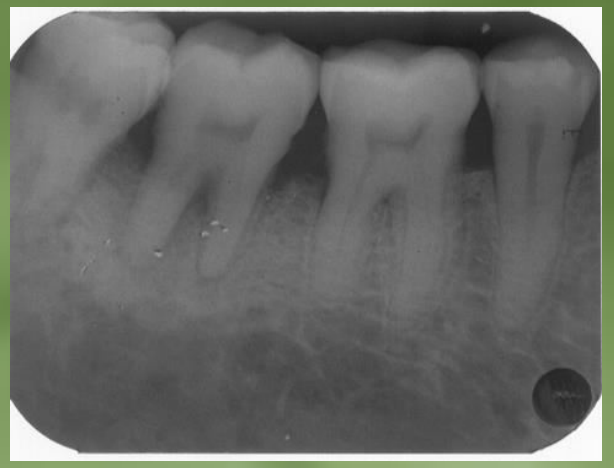


POST OPERATIVE

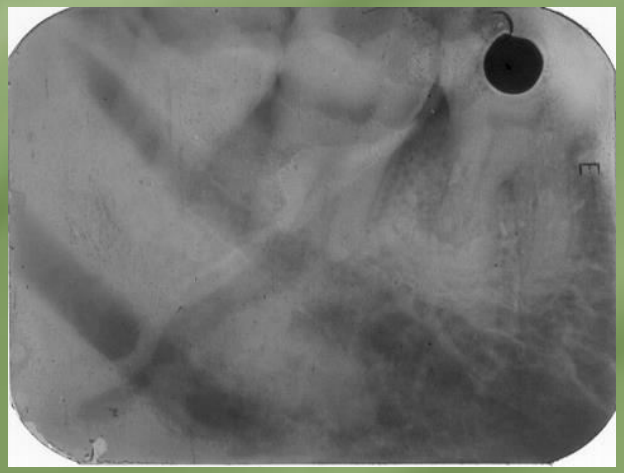
IOPA – (37) CONTROL SITE



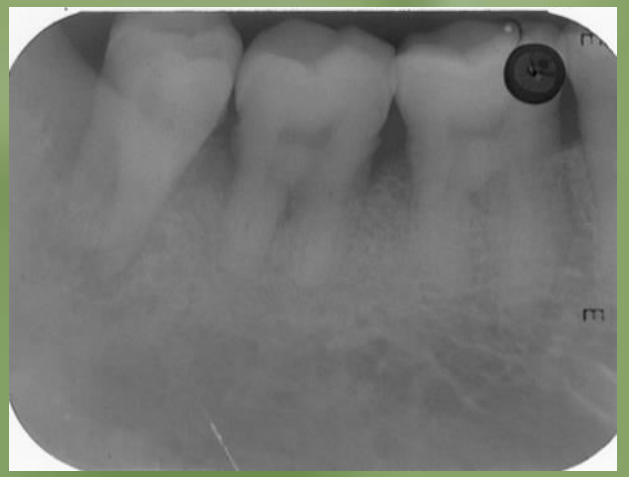
PRE OP



IMMEDIATE POST OP

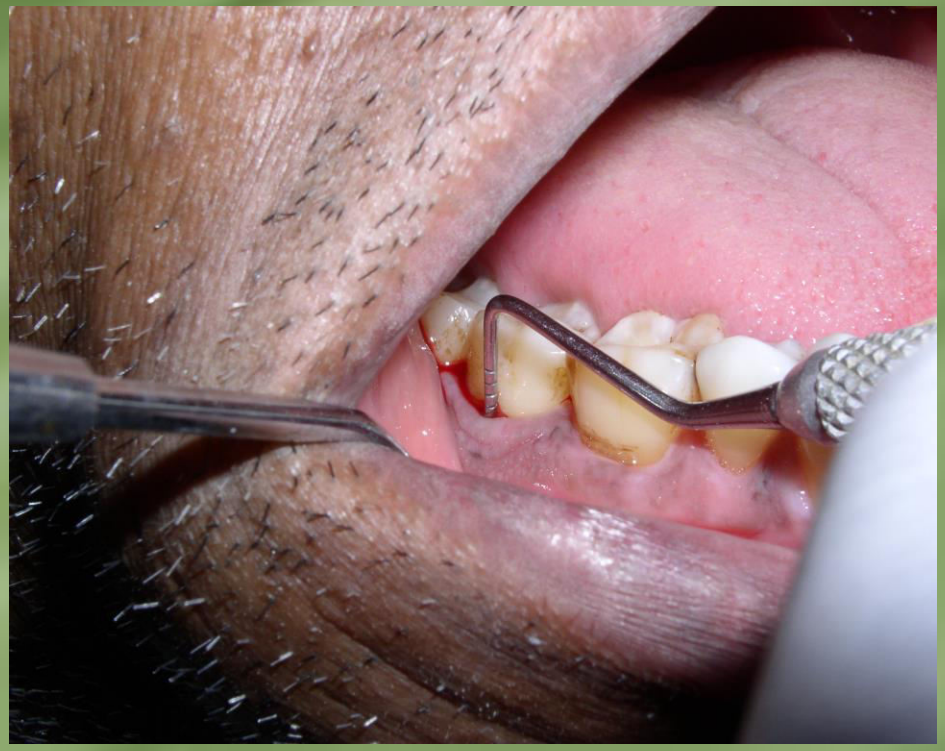


3rd MONTH

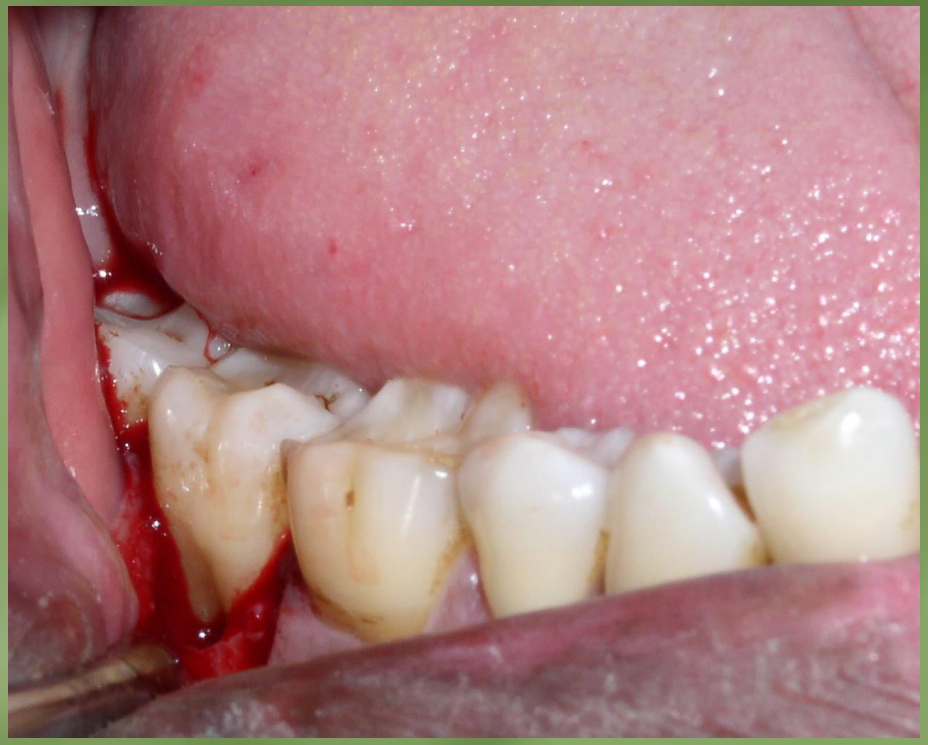


9TH MONTH

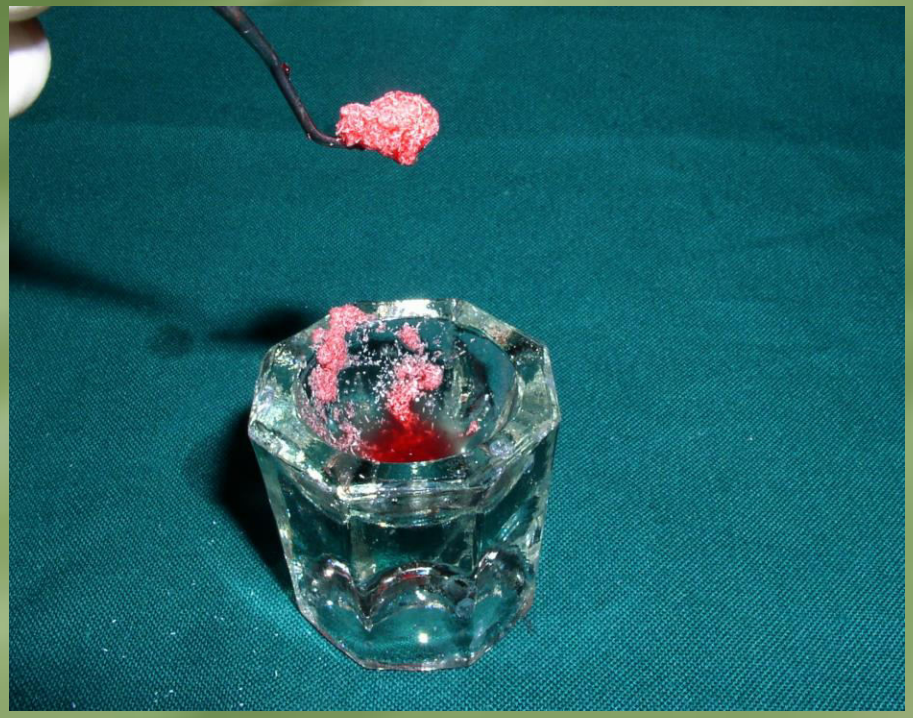
SURGICAL PROCEDURE – EXPERIMENTAL GROUP



PRE OPERATIVE

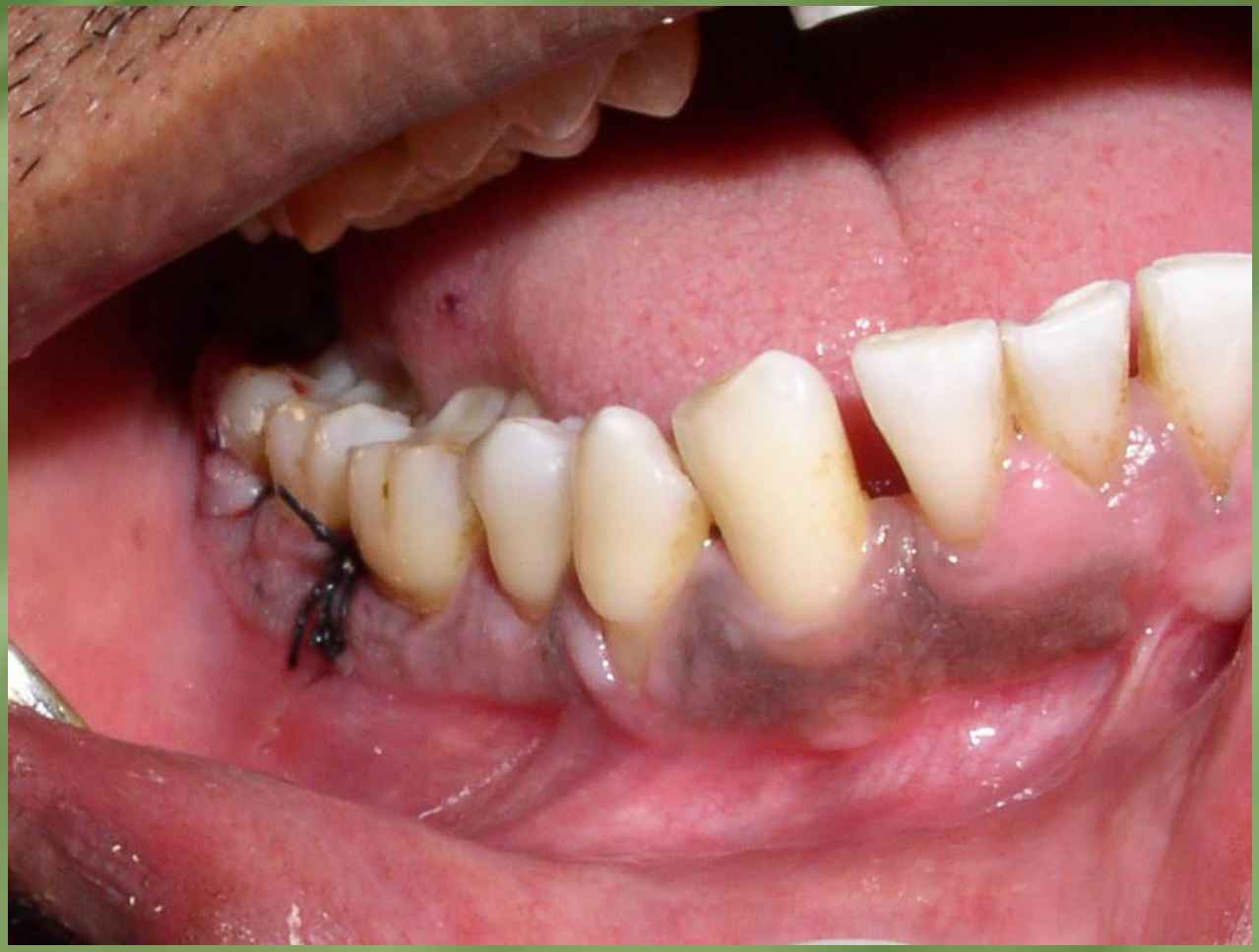


FLAP RAISED & ROOT PLANING, SOFT TISSUE DEBRIDEMENT DONE

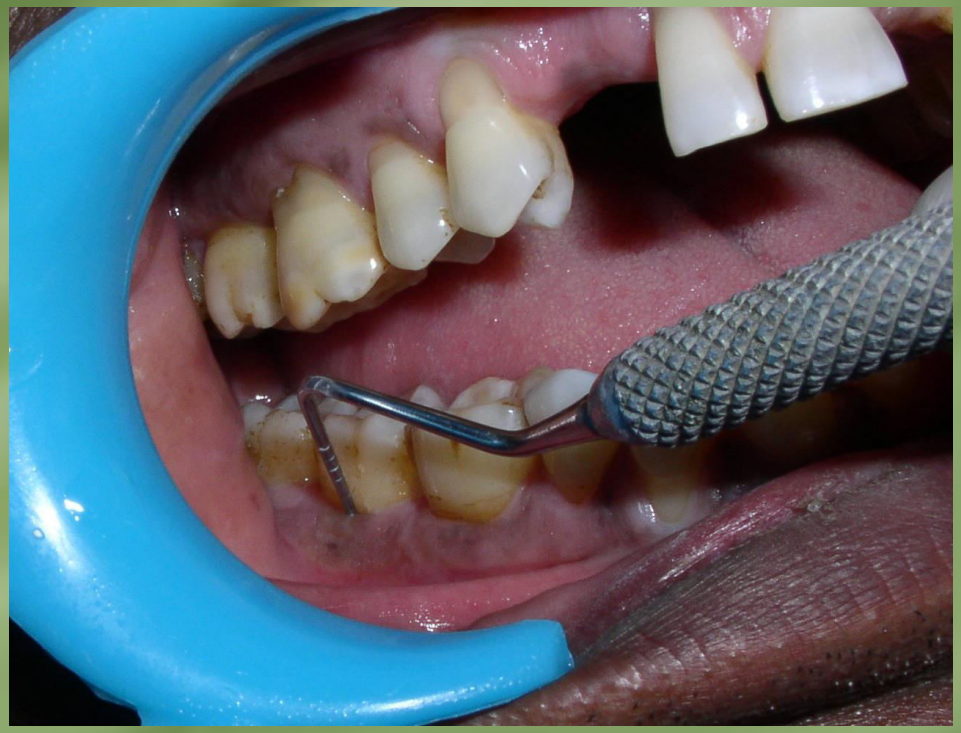


PRP/POROUS HYDROXYL -APATITE COMBINATION

GRAFT PLACED IN DEFECT



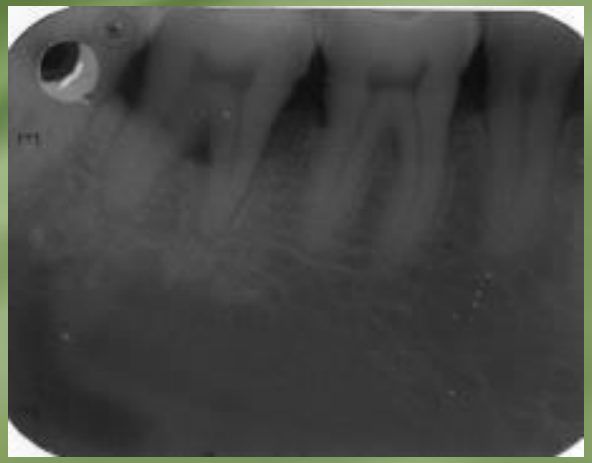
SUTURES PLACED



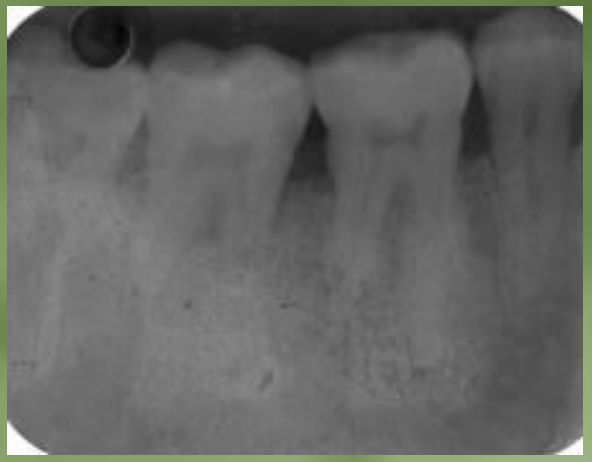
3RD MONTH
POST-OPERATIVE

9TH MONTH
POST-OPERATIVE

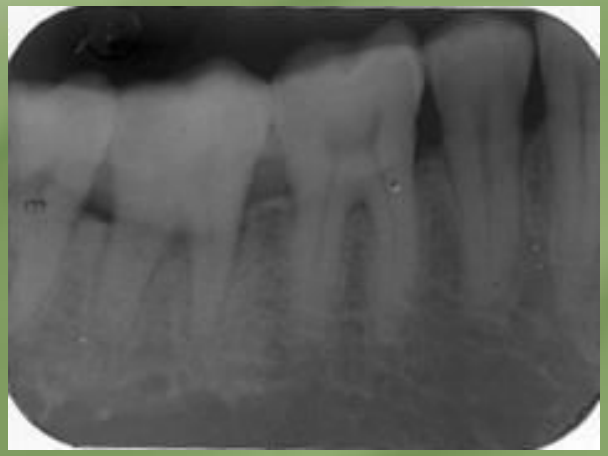
IOPA (47) EXPERIMENTAL SITE



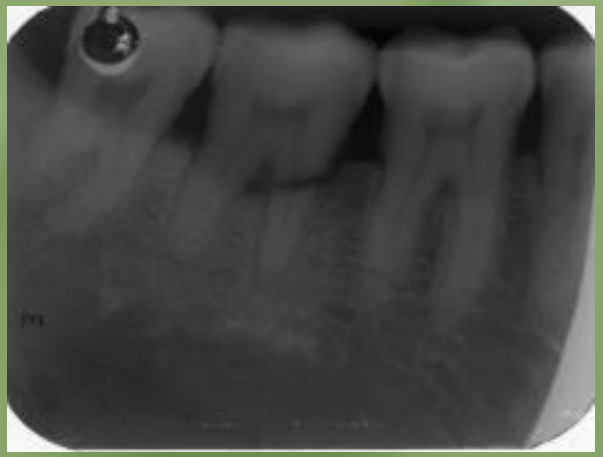
PRE OP



IMMEDIATE POST OP

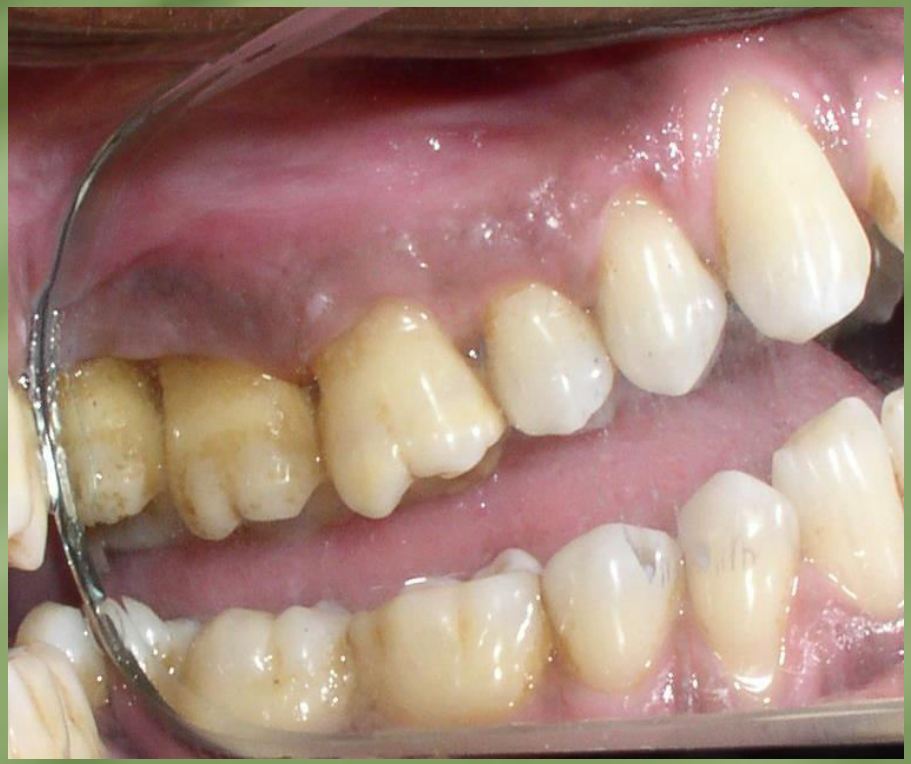


3RD MONTH POST OP

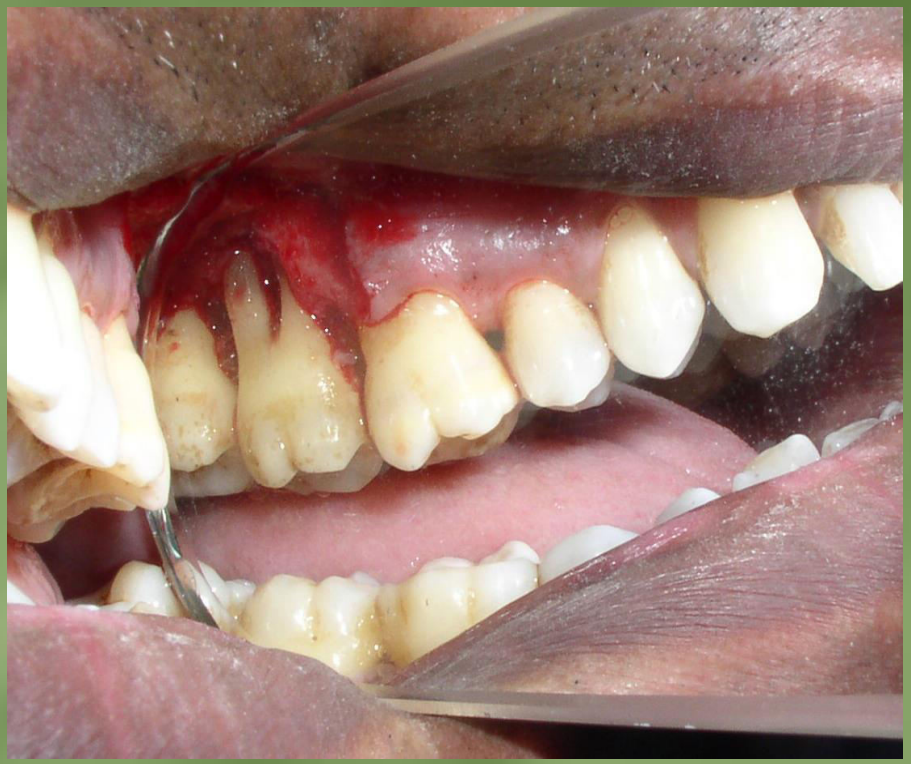


9TH MONTH POST OP

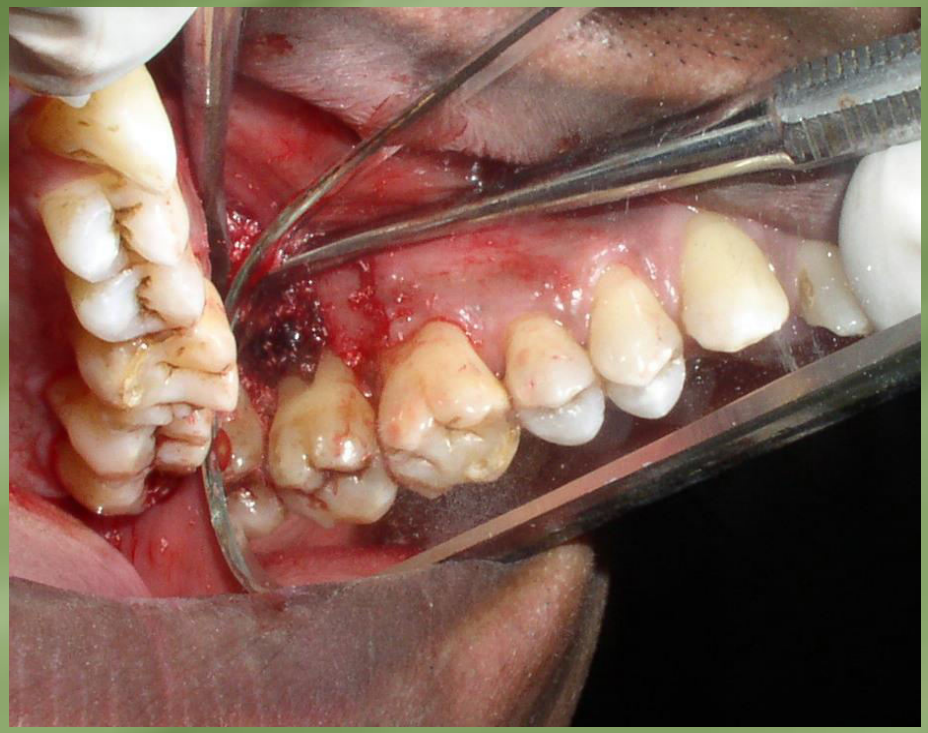
SURGICAL PROCEDURE FOR HISTOLOGICAL STUDY



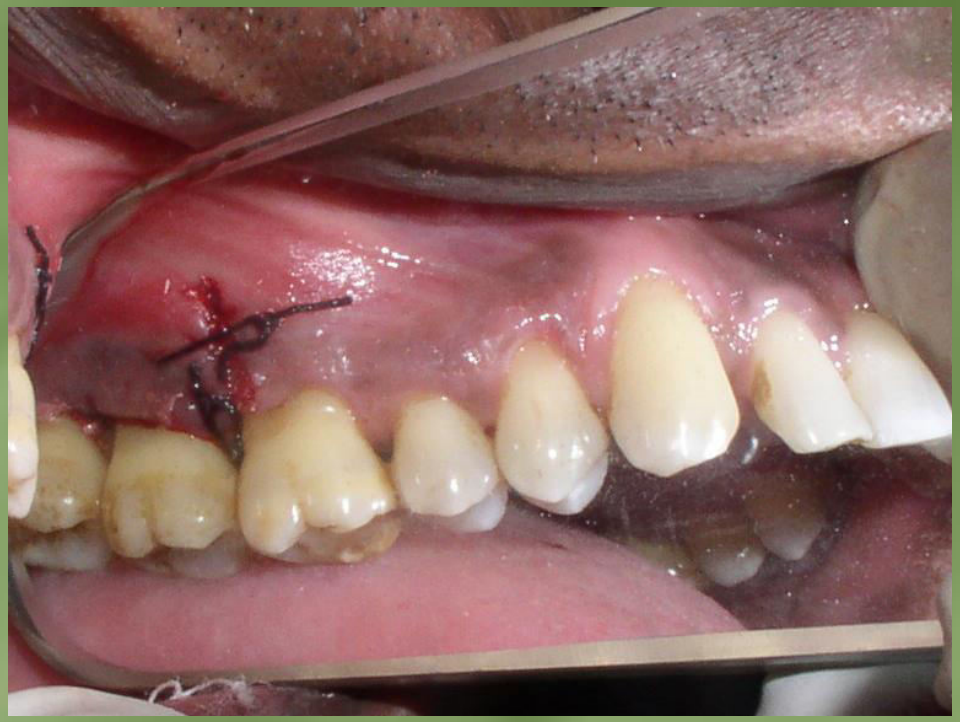
PREOPERATIVE



FLAP RAISED & ROOT PLANING, SOFT TISSUE DEBRIDEMENT DONE

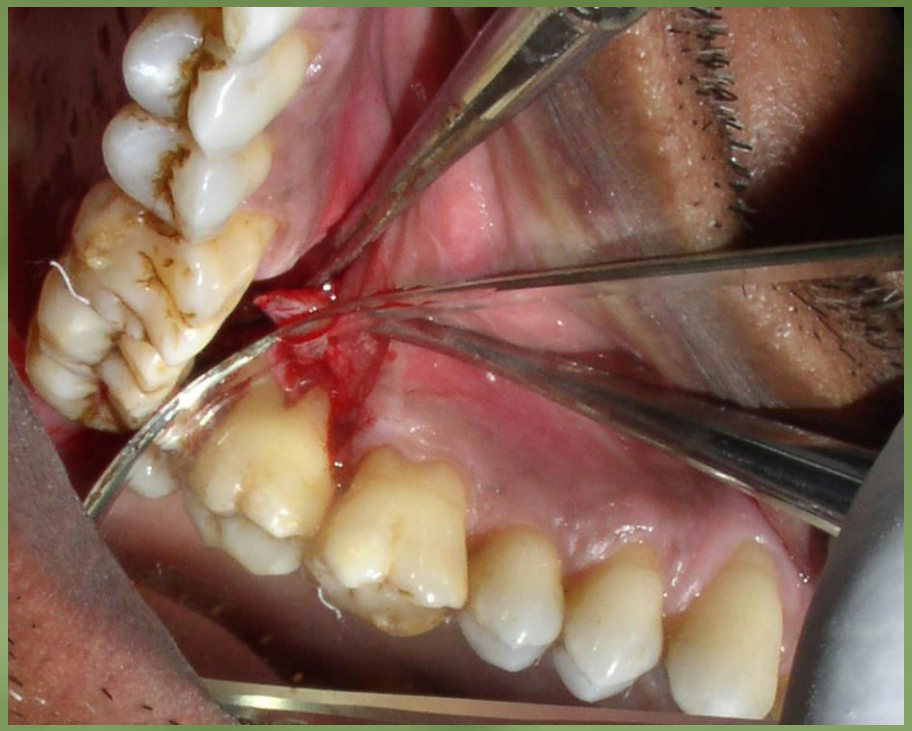


PRP/ POROUS SYNTHETIC HYDROXYLAPATITE COMBINATION PLACED AT OSSEOUS DEFECT

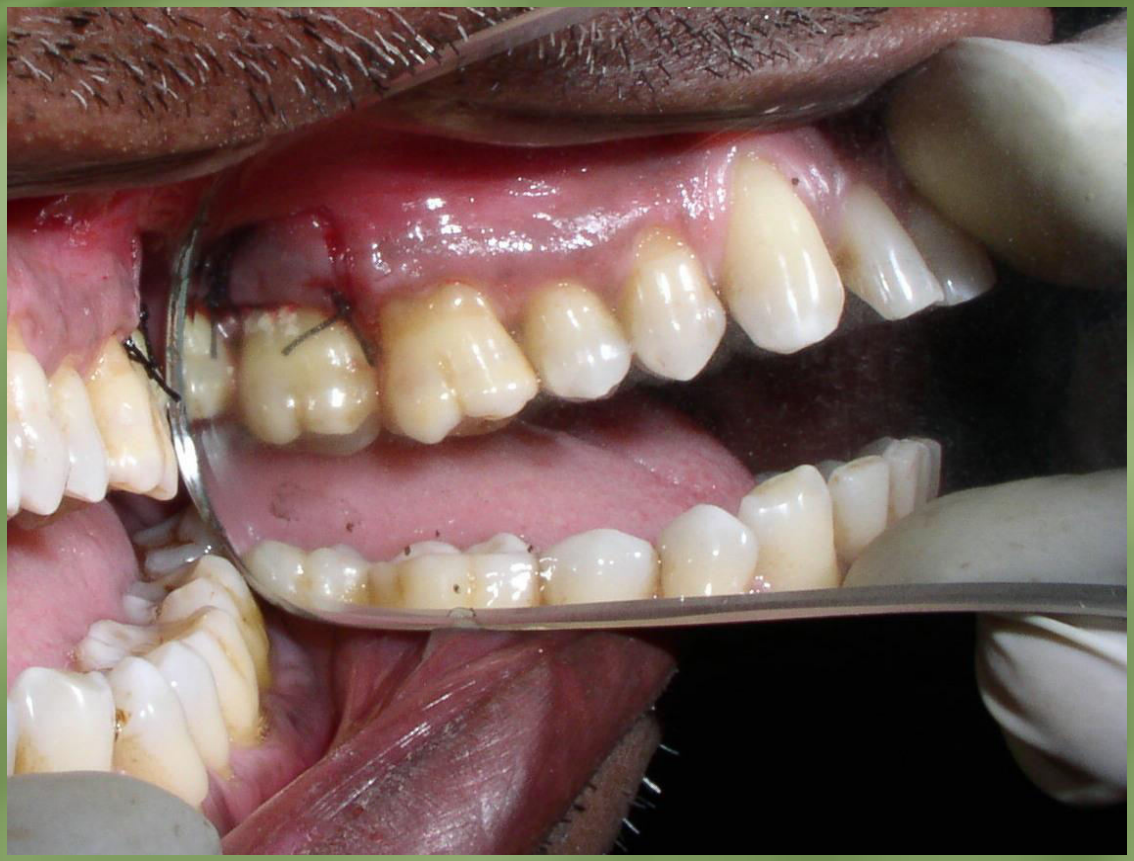


SUTURES PLACED

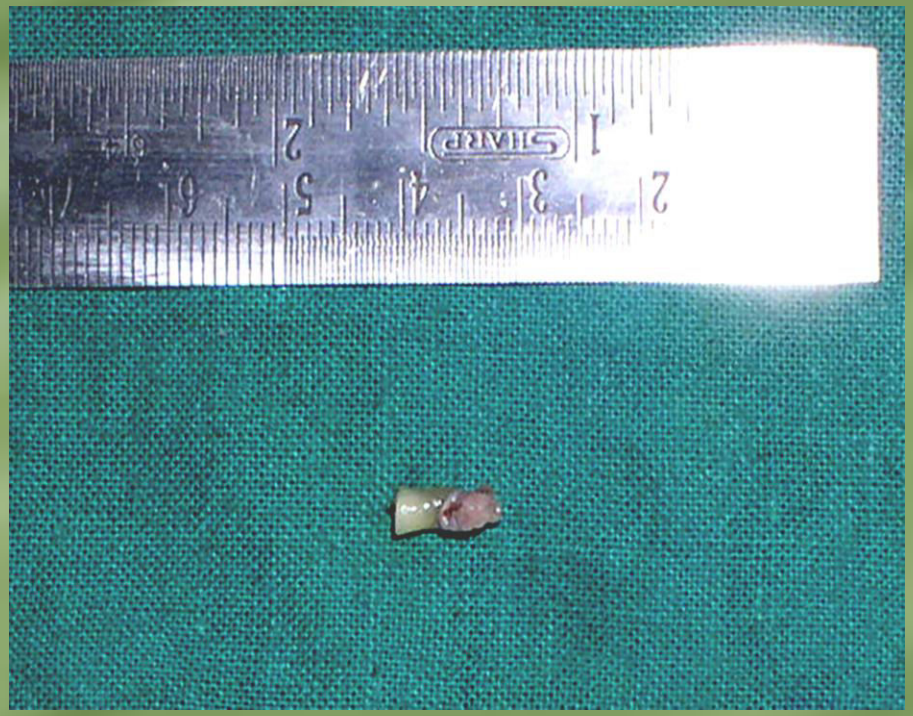
POST OPERATIVEE



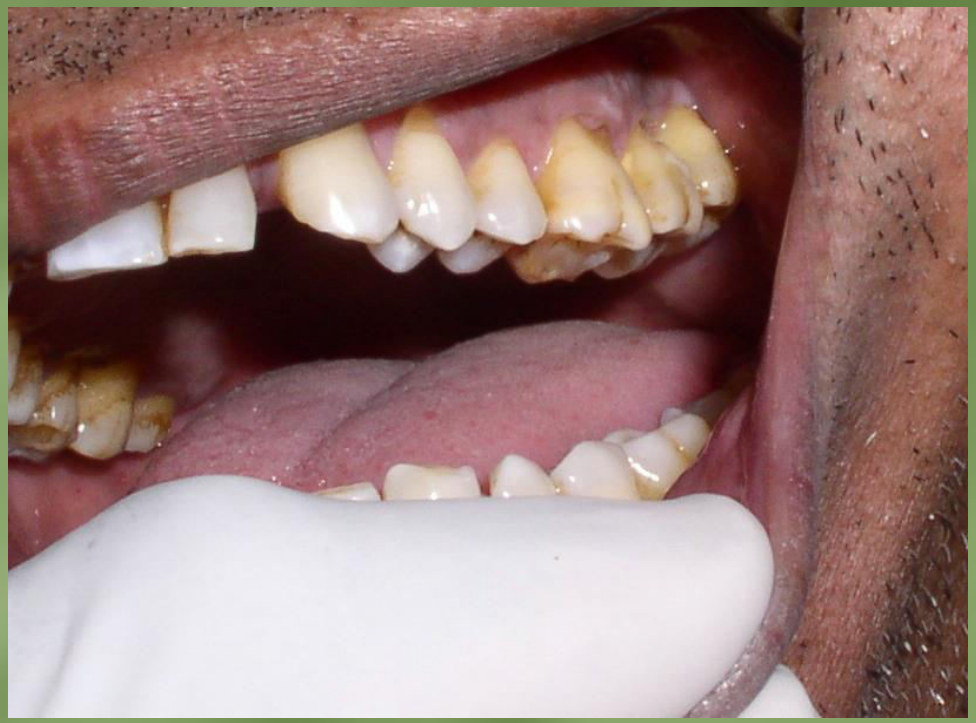
REENTRY



SUTURES PLACED

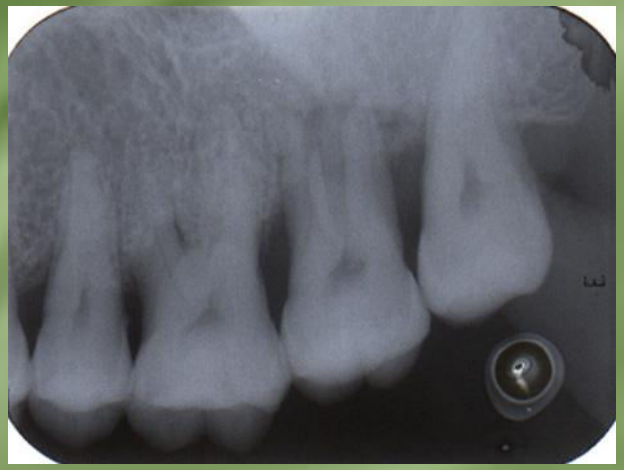


RADISECTED ROOT WITH BONE

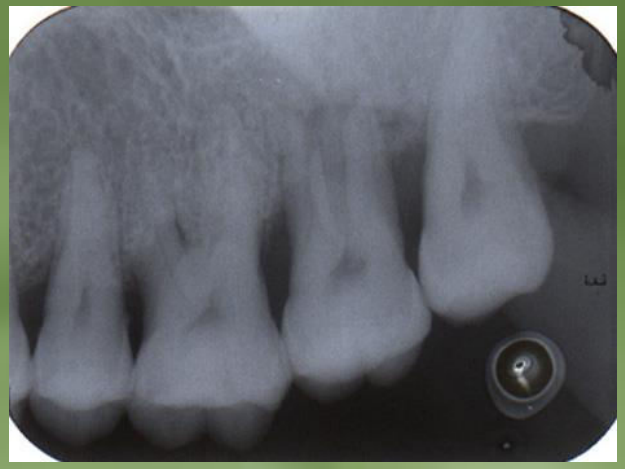


POST OPERATIVE

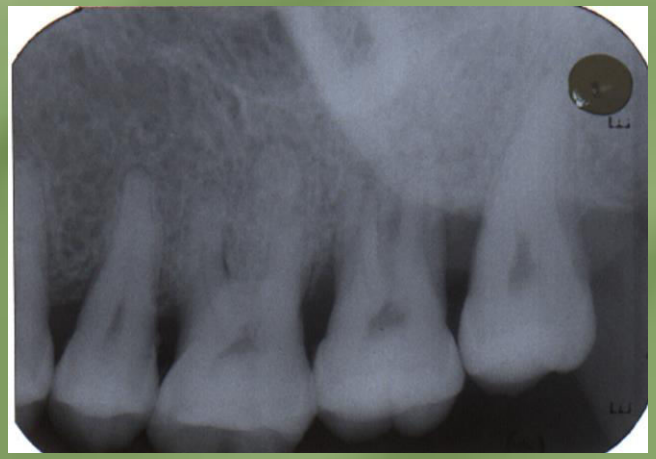
IOPA (27) HISTOLOGICAL STUDY



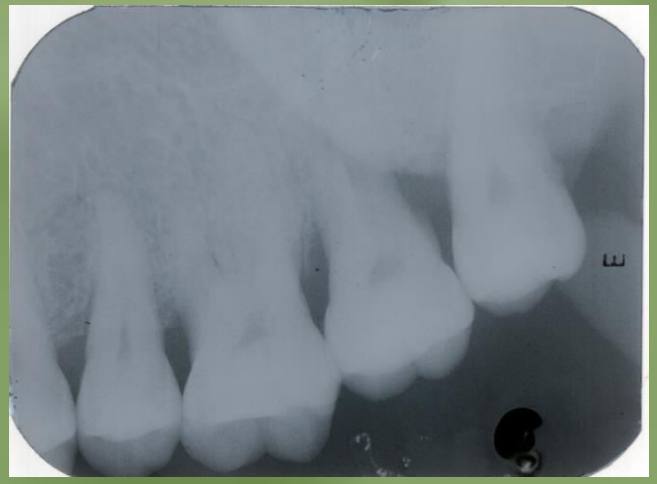
PRE OP



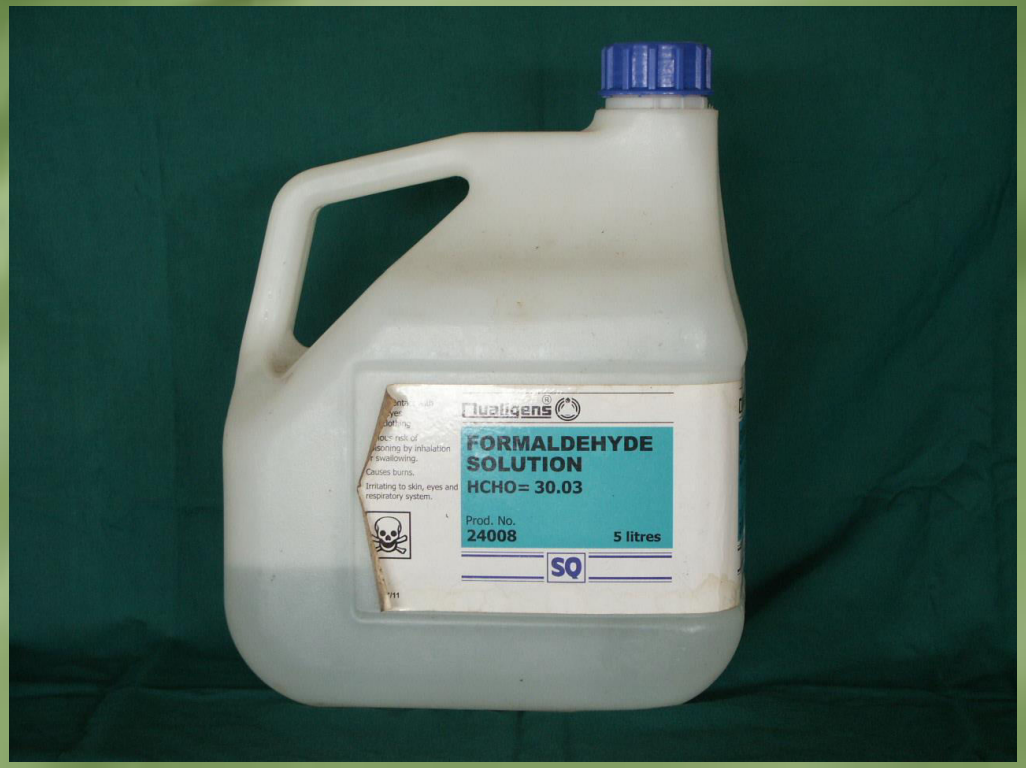
3RD MONTH POST OP



5TH MONTH



POST RADISECTION



FORMALDEHYDE SOLUTION

FORMIC ACID

SPECIMEN EMBEDDED IN PARAFFIN

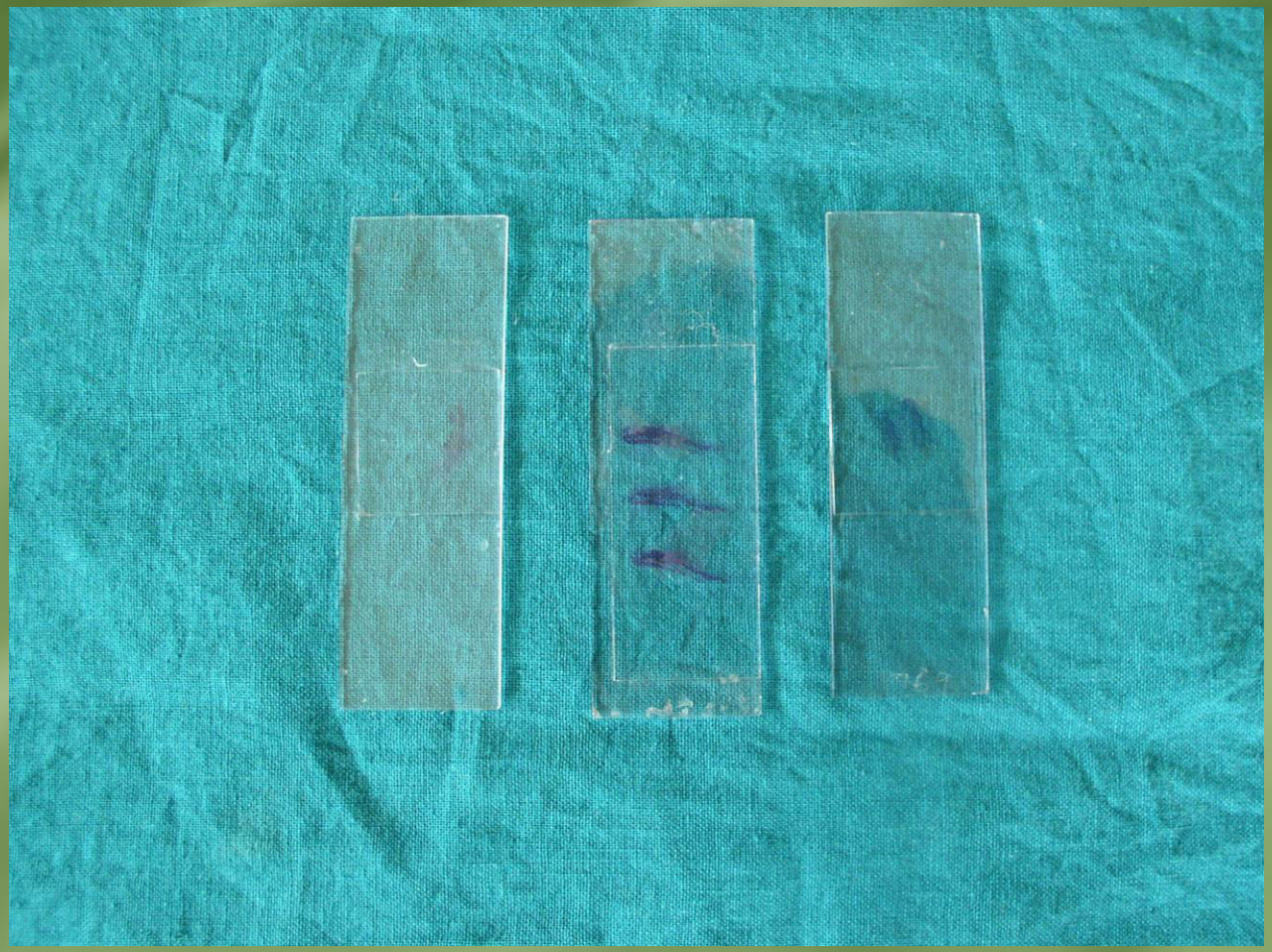
MICROTOME

WATER BATH

HOT AIR OVEN

40%, 60%, 70% and ABSOLUTE ALCHOL

HEMATOXYLIN and EOSIN STAIN



SPECIMEN MOUNTED ON SLIDE

HISTOLOGIC STUDY

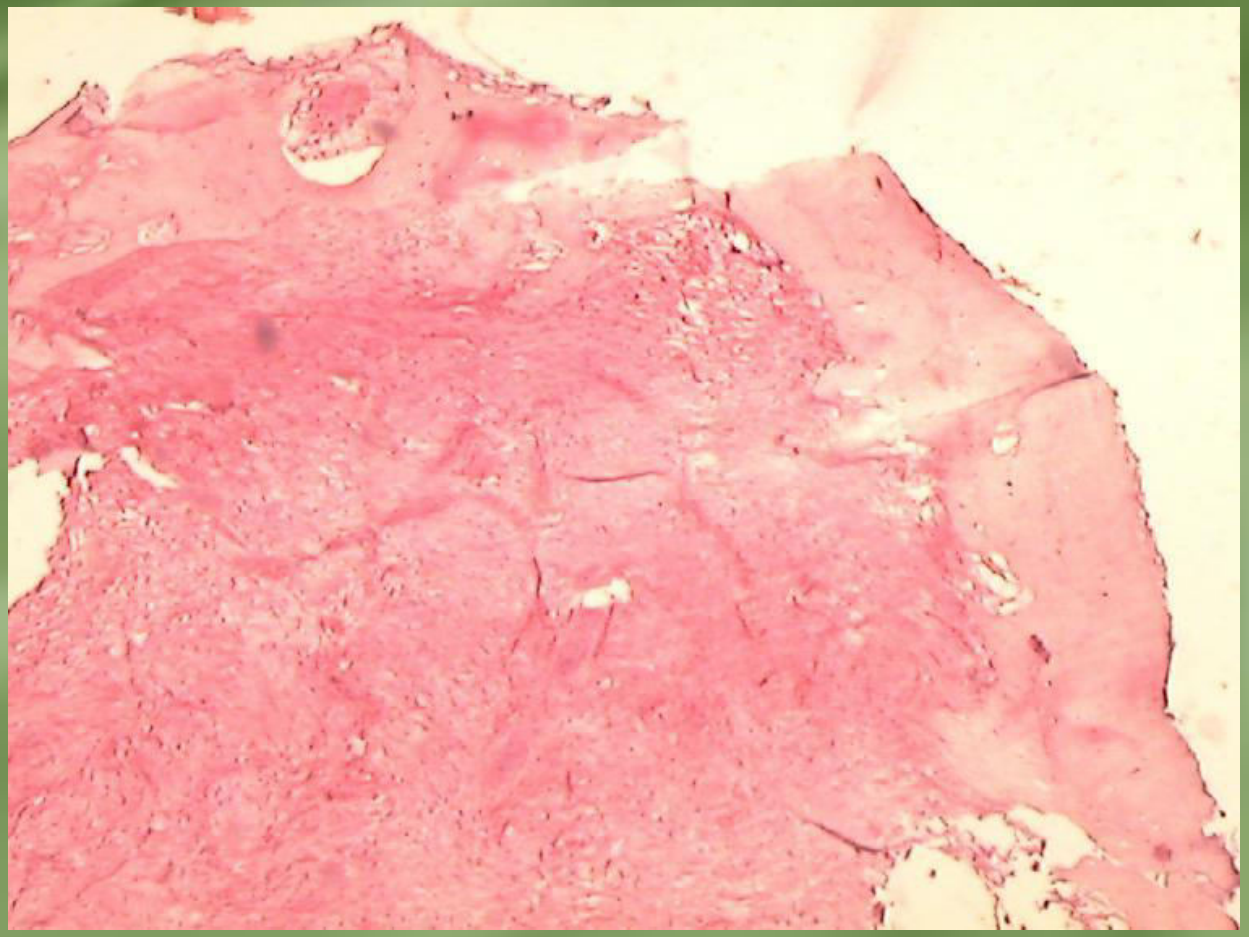


FIG.1 MAGNIFICATION = 4X
PHOTOMICROGRAPH SHOWS IMMATURE BONE WITH MATURE COLLAGEN. FEW INFLAMMATORY CELLS SEEN

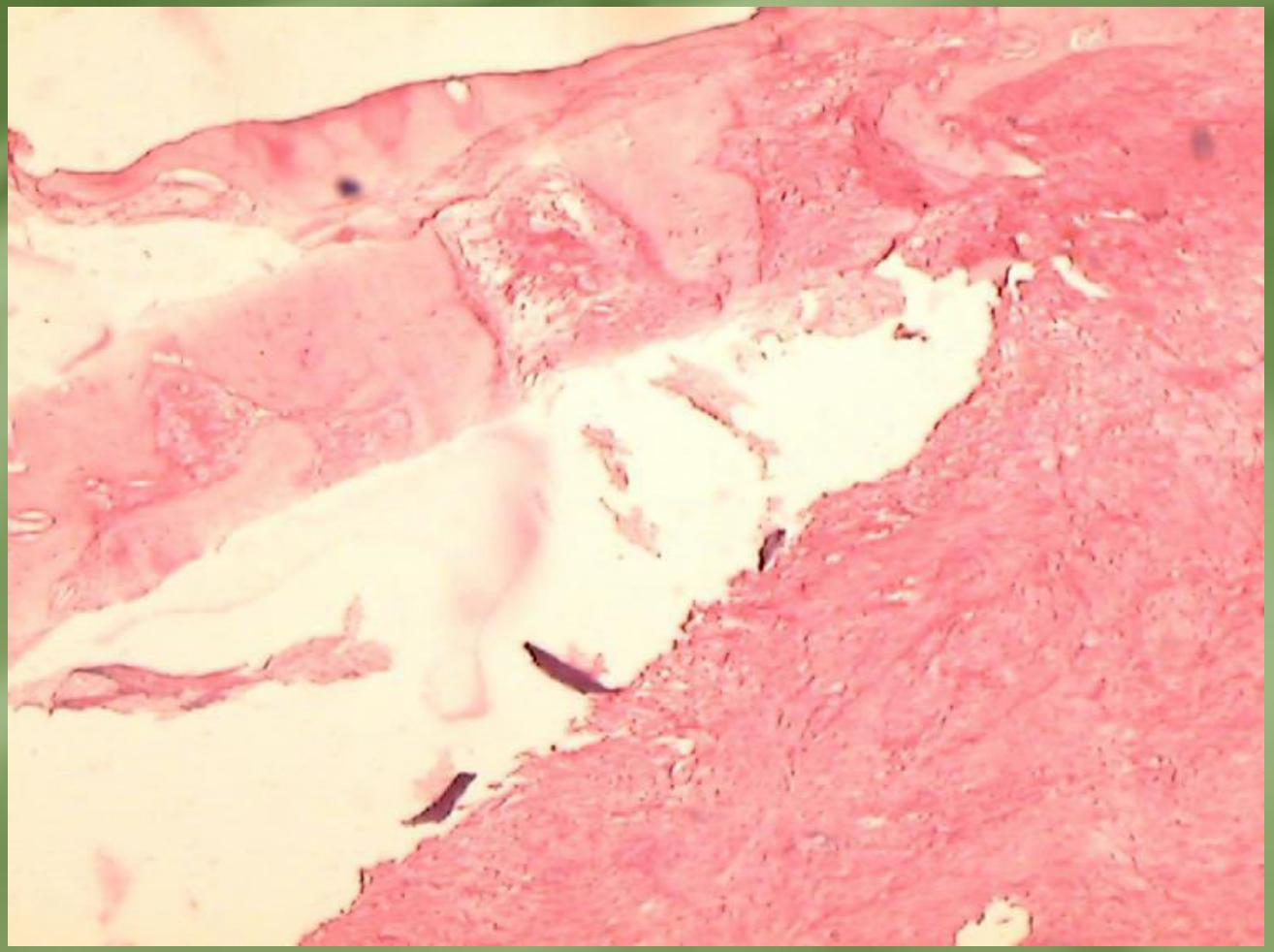


FIG. 2 MAGNIFICATION = 4X PHOTOMICROGRAPH SHOWS MATURE BONE

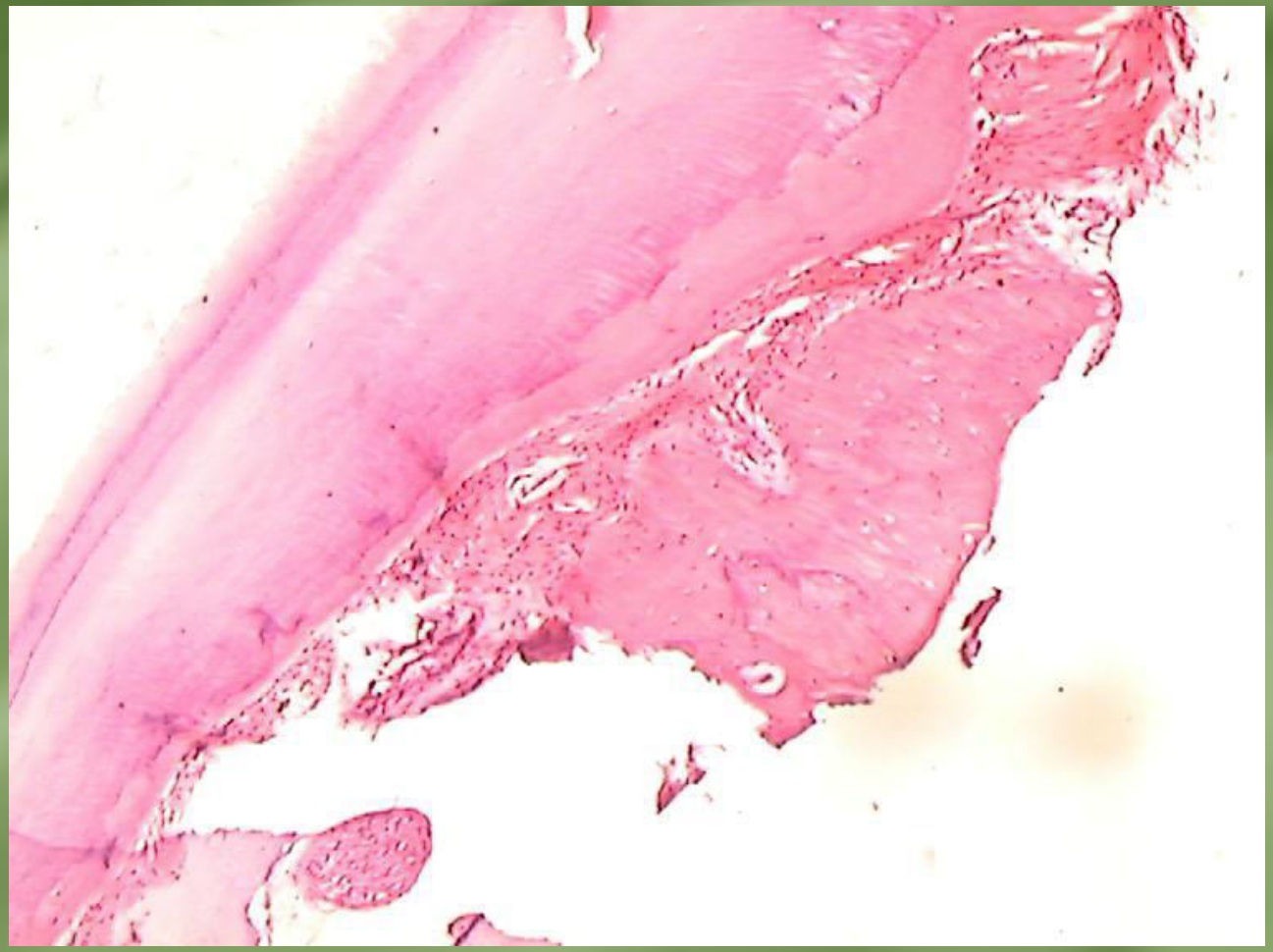


FIG.3 MAGNIFICATION = 4X PHOTOMICROGRAPH SHOWS ROOT BIT ALONG WITH PRESENCE OF CELLULAR CONNECTIVE TISSUE AND MATURE BONE

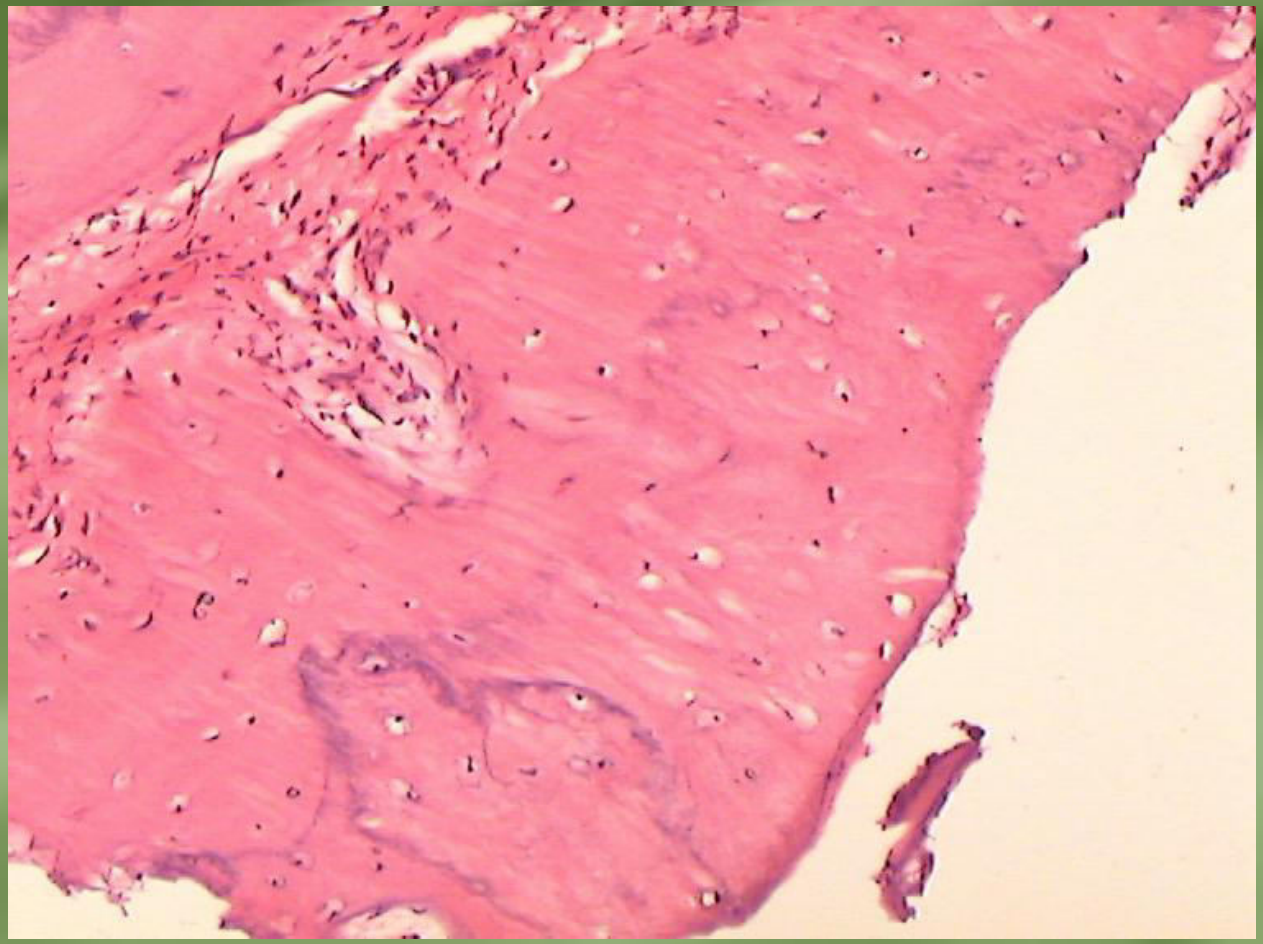


FIG.4 MAGNIFICATION = 10X PHOTOMICROGRAPH SHOWS MATURE BONE WITH OSTEOCYTES WITHIN LACUNAE AND PRESENCE OF RESTING LINES


THANK YOU