"EFFICACY OF PLATELET RICH FIBRIN IN COMBINATION WITH BIPHASIC CALCIUM PHOSPHATE IN FRACTURE HEALING"

Dissertation submitted by

DR.R.BALACHANDRAN

Dissertation is submitted to

The Tamilnadu Dr.M.G.R. Medical University, Chennai

In fulfillment of the Requirements For the award of the Degree of

MASTER OF SURGERY IN ORTHOPAEDICS

Under the guidance of

Dr.B.K.Dinakar Rai D.Ortho.,M.S.Ortho

Professor & HOD

DEPARTMENT OF ORTHOPAEDICS

PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

PEELAMEDU, COIMBATORE – 641004

TAMILNADU, INDIA

CERTIFICATE - I

This is to certify that this dissertation entitled **"EFFICACY OF PLATELET RICH FIBRIN IN COMBINATION WITH BIPHASIC CALCIUM PHOSPHATE IN FRACTURE HEALING"** is a record of bonafide research work done by **Dr.R.BALACHANDRAN** under my guidance and supervision in the Department of Orthopaedics, PSG Institute of Medical Sciences and Research, Coimbatore – 641004.

Seal and Signature of the HOD

Dr. B.K. Dinakar Rai,
Professor & HOD,
Department of Orthopaedics
PSG IMS&R, Coimbatore

Seal and Signature of the Dean

Dr. S.Ramalingam
Dean,
PSG IMS&R,
Coimbatore

CERTIFICATE – II

This is to certify that this dissertation work titled **“EFFICACY OF PLATELET RICH FIBRIN IN COMBINATION WITH BIPHASIC CALCIUM PHOSPHATE IN FRACTURE HEALING”** of the candidate **Dr.R.BALACHANDRAN** with Registration Number 221512452 for the award of Master of Surgery in the branch of Orthopaedics. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows ZERO percentage of plagiarism in the dissertation.

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To
Dr R Balachandran
Postgraduate
Department of Orthopaedics
Guides: Dr B K Dinakar Rai / Dr S M Arvind Kumar / Dr N Venkatesh Kumar
PSG IMS & R
Coimbatore

Ref: Project No.15/424

Date: December 30, 2015,

Dear Dr Balachandran,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 24.12.2015 to conduct the research study entitled *"Efficacy of platelet rich fibrin in combination with biphasic calcium phosphate in fracture healing"* during the IHEC review meeting held on 28.12.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Version 1 dated 24.12.2015)
3. Informed consent forms (Version 1 dated 24.12.2015)
4. Data collection tool (Version 1 dated 24.12.2015)
5. Current CVs of Principal investigator, Co-investigator
6. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 28.12.2015 at Research Conference Room, PSG IMS & R between 10.00 am and 12.30 pm:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mrs Y Ashraf	MPT	Physiotherapy	Female	Yes	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	No
4	Dr A Jayavardhana	MD	Clinician (Paediatrics)	Male	Yes	Yes
5	Mr P Karuppuchamy	M Phil in PSW	Social Scientist	Male	Yes	Yes
6	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	Yes
7	Mr. R. Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes

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8	Dr. Parag K Shah	DNB	Clinician (Ophthalmology)	Male	No	No
9	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	Yes
10	Mrs P Rama	M Pharm	Non-Medical (Pharmacy)	Female	Yes	Yes
11	Dr. Seetha Panicker (Vice-chairperson, IHEC)	MD	Clinician (Obstetrics & Gynaecology)	Female	Yes	Yes
12	Dr R Senthil Kumar	MD	Clinician (Endocrinology)	Male	Yes	Yes
13	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
14	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	Yes
15	Mrs. Swasthika Soundararaj	MBA	Lay person	Female	No	Yes
16	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
    a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
    b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
    c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
    d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
    e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented

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f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review

7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

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To
Dr R Balachandran
Postgraduate
Department of Orthopaedics
Guide/s: Dr B K Dinakar Rai / Dr S M Arvind Kumar / Dr N Venkatesh Kumar
PSG IMS & R
Coimbatore

Ref: Project No. 15/424

Date: October 6, 2017

Dear Dr Balachandran,

Institutional Human Ethics Committee, PSG IMS&R discussed your request to amend the study entitled "*Efficacy of platelet rich fibrin in combination with biphasic calcium phosphate in fracture healing*" during the IHEC review held on 22.09.2017.

The following documents were reviewed and approved:

1. Your letter dated 09.09.2017
2. Amendment reporting form dated 09.09.2017
3. Study proposal (Version 2 dated 09.09.2017)

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 22.09.2017 at College Council Room, PSG IMS & R between 2.30 pm and 4.30 pm:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mrs Y Ashraf	MPT	Physiotherapy	Female	Yes	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	No
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13	Mrs. Swasthika Soundararaj	MBA	Lay person	Female	No	Yes
14	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The Committee approves your request to remove Group B from the study.

The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
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7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Thanking You,

Yours Sincerely,

SECRETARY
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Dr D Vijaya
Member-Secretary
Institutional Human Ethics Committee

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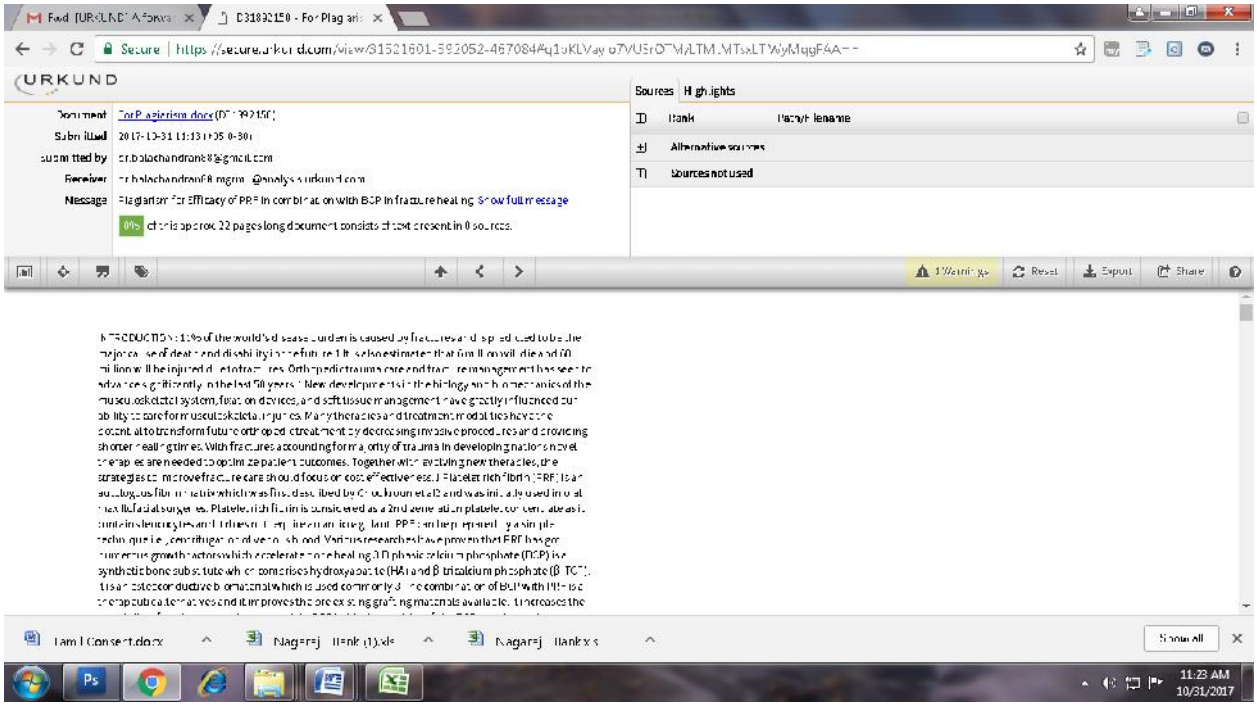
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DECLARATION

I Hereby declare that this dissertation entitled **"EFFICACY OF PLATELET RICH FIBRIN IN COMBINATION WITH BIPHASIC CALCIUM PHOSPHATE IN FRACTURE HEALING"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.B.K.Dinakar Rai, D.Ortho.,M.S. Ortho**, Professor & HOD**,** Department of Orthopaedics, PSG IMS&R, Coimbatore, dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University in fulfillment of the university regulations for the award of degree of Master of Surgery in Orthopaedics. This dissertation has not been submitted for award of any other degree or diploma.

Signature of the Candidate

Dr.R.BALACHANDRAN

ACKNOWLEDGEMENT

At the outset, I thank the god for giving me the strength to perform all my duties.

It is indeed a great pleasure to recall the people who have helped me in the completion of dissertation. Naming all the people who have helped me achieving this goal would be impossible, yet I attempt to thank a selected few who have helped me in diverse ways.

I acknowledge and express my humble gratitude and sincere thanks to my beloved teacher and guide Dr. B. K. Dinakar Rai ., D.Ortho., M.S. Ortho, Professor & HOD, Department of Orthopaedics, PSGIMS&R, Coimbatore for his valuable suggestion, guidance, great care and attention to details, that he has so willingly shown in the preparation of this dissertation.

I owe a great deal of respect and gratitude to my professor, Dr. Arvind Kumar M.S.Ortho for his whole hearted support in completion of this dissertation.

I owe a great deal of respect and gratitude to my professor, Dr. N.Venkatesh Kumar D.Ortho, DNB., for his whole hearted support in completion of this dissertation.

I also owe a great deal of respect and gratitude to my Associate professors Dr.Prasanna C M.S (Ortho)and Dr. Sandeep Maran for their whole hearted support in completion of this dissertation.

I also express my sincere thanks to my Assistant professors Dr. Yeseswi Tellakula and Dr. Sajjad Ali Department of Orthopaedics, PSGIMS&R, Coimbatore for their timely suggestions and all round encouragement.

I am immensely indebted to my parents for their continuous support without them this study could not have been reality.

My sincere thanks to the OP staff, Post graduate colleagues and my friends for their whole hearted support.

Finally I thank my patients who formed the backbone of this study without whom this study would not have been possible

CONTENTS

LIST OF ABBREVIATIONS

PRF	Platelet-Rich Fibrin
BCP	Biphasic Calcium Phosphate
VEGF	Vascular endothelial growth factor
PDGF	Platelet derived growth factor
HA	Hydroxyapatite
β-TCP	β-tricalcium phosphate
PAS	Periodic acid-Schiff
AO	Arbeitsgemeinschaft fur Osteosynthesefragen
IGF	Insulin like growth factor
TGF β	Transforming growth factor β
CFU-F	Colony forming unit fibroblasts
MSC	Mesenchymal stem cells
PRP	Platelet rich plasma
PC	Platelet concentrates
PPP	Platelet poor plasma
cPRP	Concentrated Platelet rich plasma

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ABSTRACT

INTRODUCTION

11% of the world's disease burden is caused by fractures and is predicted to be the major cause of death and disability in the future.[1]The combination of biphasic calcium phosphate (BCP) with platelet rich fibrin (PRF) is a therapeutic alternative and it improves the pre-existing grafting materials available. The study was conducted to prove the efficacy of PRF + BCP in fracture healing process. [1]

This study's aim was to compare the efficacy of platelet rich fibrin in combination with biphasic calcium phosphate in early callus formation.

MATERIALS AND METHODS

A total of 26 patients participated in this study. They were divided into two groups, Group A (Study group) patients with upper limb diaphyseal fractures underwent open reduction and internal fixation with application of PRF + BCP over the fracture site which was compared with a control group where the patients received only current standard of care. Post operatively fracture healing was assessed using X rays. X rays were taken every month and the time taken for appearance of callus in the X rays were considered to be the beginning of fracture healing process. If the healing does not occur till 5 months, it will be considered to be non-union.

RESULTS

76.9% of the patients who received PRF+BCP showed callus formation within the sixth week, whereas only 38.5% of the controls showed callus formation during their twelfth week. It was found to be statistically significant ($p<0.05$).

CONCLUSION

92.3% of the cases showed callus formation in a period of less than 8 weeks whereas 46.2% of the controls showed callus formation only during their 12^{th} week only. This shows that, PRF+BCP favours early callus formation and improves fracture healing process.

KEYWORDS

Biphasic calcium phosphate; Platelet rich fibrin; Callus formation; Fracture healing; Upper limb diaphyseal fractures.

INTRODUCTION

11% of the world's disease burden is caused by fractures and is predicted to be the major cause of death and disability in the future.[1]It is also estimated that 6 million will die and 60 million will be injured due to fractures. Orthopedic trauma care and fracture management has seen to advance significantly in the last 50 years.[1]

New developments in the biology and biomechanics of the musculoskeletal system, fixation devices, and soft tissue management have greatly influenced our ability to care for musculoskeletal injuries. Many therapies and treatment modalities have the potential to transform future orthopedic treatment by decreasing invasive procedures and providing shorter healing times. With fractures accounting for majority of trauma in developing nations, novel therapies are needed to optimize patient outcomes. Together with evolving new therapies, the strategies to improve fracture care should focus on cost effectiveness.[1]

Platelet Rich Fibrin(PRF) is an autologous fibrin matrix which was first described by Choukroun et al[2] and was initially used in oral maxillofacial surgeries. Platelet Rich Fibrin is considered as a 2nd generation platelet concentrate as it contains leucocytes and it does not require an anticoagulant. PRF can be prepared by a simple technique ie, centrifugation of venous blood. Various researches have proven that PRF has got numerous growth factors which accelerate bone healing.[3]

Biphasic Calcium Phosphate(BCP) is a synthetic bone substitute which comprises Hydroxyapatite(HA) and β-tricalcium phosphate(β-TCP). It is an osteoconductive biomaterial which is used commonly.[3]

The combination of BCP with PRF is a therapeutic alternative and it improves the pre-existing grafting materials available. It increases the versatility of the bone substitute materials. PRF holds the particles of the BCP together acting as a biological adhesive. Thus helping its manipulation easier and it also provides the survival and vascularization of the graft.[3]

There are animal studies where PRF is augmented in bone.[2,3] But there are not much human studies conducted in Orthopaedics using PRF + BCP augmentation. Thus the study was conducted to prove the efficacy of PRF + BCP in fracture healing process.

AIM OF THE STUDY

To evaluate the efficacy of PRF in combination with BCP in fracture healing.

OBJECTIVES

To assess the rate of early callus formation in study group and to compare it with a control group.

REVIEW OF LITERATURE

ANATOMY OF BONE

Based on the shape, bones are classified into four types ie. long, short, flat and irregular. A typical long bone usually comprises of two ends or epiphyses and a portion in the intermediate called the shaft or diaphysis. The part of the shaft which joins the epiphysis is called metaphysis. The growth cartilage is present as a thin plate one at each end separating the metaphysis from the epiphysis, this is called the epiphyseal plate. During the time of maturity, the epiphysis fuses with the metaphysis and the epiphyseal plate gets replaced by bone. The articular cartilage covers the articular ends of the epiphysis. Periosteum covers the rest of the bone providing attachment to the muscles, tendons and ligaments etc., Sharpey's fibres are the strands of fibrous tissue that connects the bone to the periosteum.[4,6]

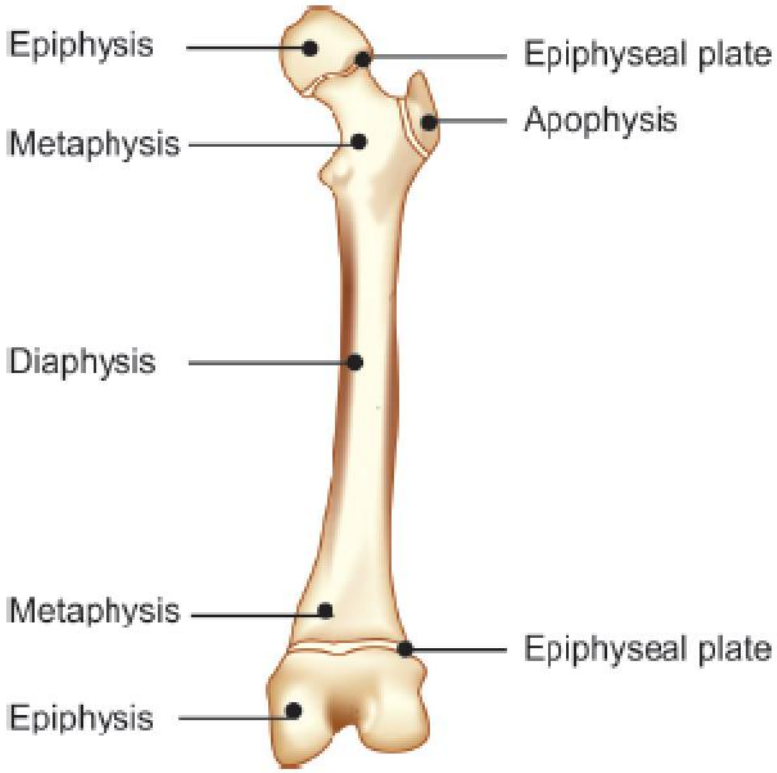


FIGURE I: PARTS OF LONG BONE

Microscopically, bone is classified into two types ie, woven or lamellar. Woven bone also known as immature bone is characterized by random arrangement of collagen fibres and osteocytes. Lamellar bone also known as mature bone has an orderly arrangement of collagen fibres and bone cells. Lamellar bone constitutes of all the bones both cancellous and cortical. The lamellae is densely packed in cortical bone whereas it is loosely packed in cancellous bone[6].

Osteon is the basic structural unit of a lamellar bone which consists of a series of concentric laminations or lamellae surrounded by a central canal called

the Haversian canal. The Haversian canal runs longitudinally and is connected with each other and also with Volkmann's canal. The Volkmann's canal runs horizontally from endosteal to periosteal surfaces. Shaft of a bone comprises of cortical bone, and the ends are made up of cancellous bone. The junction formed by the two is termed as cortico-cancellous junction which is more prone to fractures[5].

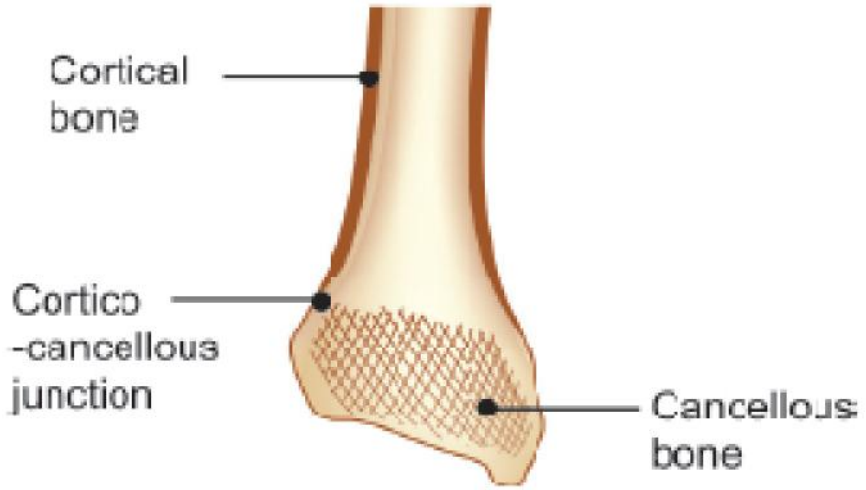


FIGURE II: CORTICOCANCELLOUS JUNCTION

COMPOSITION OF BONE[5,6]

Bone is comprised of bone cells and extracellular matrix. The matrix is made up of organic and inorganic materials. The organic matrix comprises of collagen which constitutes of 30-35% of dry weight in the bone. The inorganic matrix primarily comprises of phosphorus and calcium salts, especially hydroxyapatite which constitutes about 65-70% of dry weight of a bone.

Three different types of cells are seen in the bone. They are:

1) *Osteoblast:* These are cells that are rich in alkaline phosphatase, phosphorylases and glycolytic enzymes. These are involved in ossification
2) *Osteocytes:* These are cells that are rich in PAS positive granules and glycogen. Osteocytes are mature bone cells which differ in activity and they take up the form of a reticulocyte or osteoclast.
3) *Osteoclasts:* They consist of glycolytic acid hydrolases, acid phosphatase enzymes and collagenases. These consist of multi-nucleate mesenchymal cells that are involved in bone resorption.

GROWTH OF A BONE[4,5,6]

Clavicle is the only long bone that develops by enchondral ossification (develops from cartilage). In this type the primary centre of ossification commences from the shaft before birth. The secondary centre of ossification occurs from the epiphyses which appear at the ends of the bone after birth.

Growth of a bone occurs lengthwise in a continuous manner in the epiphyseal plate. Subperiosteal new bone deposition results in increase in the bone girth.

During the end of growth period, the growth ceases by fusion of epiphyses with diaphysis. Apophysis are the secondary centres of ossification that does not contribute to the length of a bone.

BLOOD SUPPLY OF BONES[4]

Blood supply of a long bone is usually derived from the following:

1) *Nutrient artery:* These enter around the middle of the bone and divides into two branches, each of them running towards the end of the bones which further divides into parallel vessels that runs towards the metaphysis.
2) *Metaphyseal vessels*: These are small vessels deriving from the anastomosis around the joint, which pierce the metaphysis along the attachment of the joint capsule.

3) *Epiphyseal vessels*: These vessels enter the epiphysis directly.

4) *Periosteal vessels*: Periosteum consists of rich blood supply. Numerous little vessels enter the bone from these vessels. Outer 1/3rd of the bone is supplied by periosteal vessels whereas inner 2/3rd is supplied by nutrient artery.

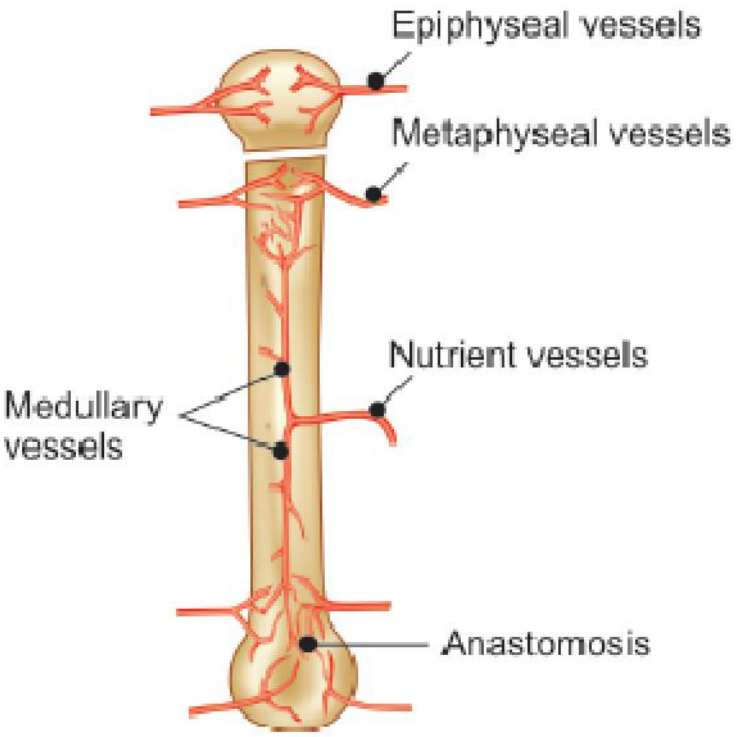


FIGURE III: BLOOD SUPPLY OF BONES

FRACTURE[1,4]

A break in the continuity of a bone is called a fracture. Injury to the musculoskeletal system results in damage to the bones, joints, tendons and muscles. It may also damage the neurovascular bundle. Depending on the aetiology, the fracture's relationship to the external environment, pattern of the fracture and displacement of the fracture, fractures are classified into various types:

BASED UPON AETIOLOGY

Traumatic fractures: A fracture caused by a trauma is called traumatic fracture. Normally bone can withstand a considerable force, but when it is subjected to an excessive force it breaks. E.g: fight, road traffic accident and fractures caused by a fall.

Pathological fractures: An underlying disease which causes a fracture is called a pathological fracture. No force is required to cause a fracture like this. Pathological fractures very often go to non-union.

Stress fracture: A chronic repetitive injury sustains a special type of fracture called stress fracture. This presents with only pain and x-rays may not show these fractures.

BASED UPON DISPLACEMENT

Undisplaced fracture: This fracture does not have any displacement. This is not that easy to identify.

Displaced fracture: These have significant displacement and are easy to identify. The factors that are responsible for displacement are:

1) The fracturing force
2) Pull of the muscle on the fracture fragments
3) The gravity

The distal fragment's displacement in relation to the proximal fragment is used to describe the displacement of a fracture. Displacement can be in form of angulation, shift or rotation.

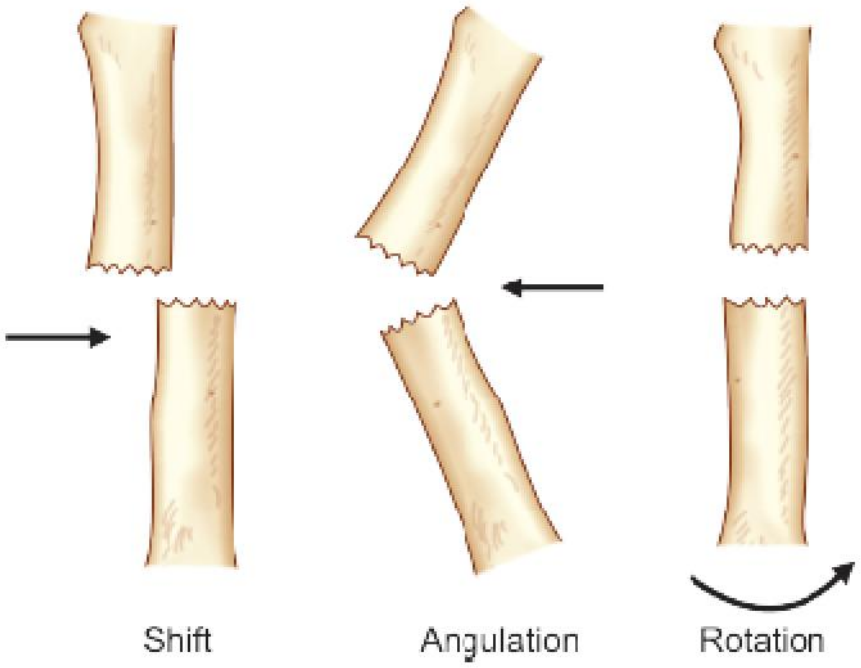


FIGURE IV: FRACTURE DISPLACEMENT

BASED ON THE RELATIONSHIP WITH EXTERNAL ENVIRONMENT

Closed fracture : It's a fracture that does not communicate with the external environment i.e., the overlying soft tissues and skin will be intact.

Open fracture : Fracture with break in the overlying soft tissues and skin resulting in the communication to the external environment, is called an open fracture. It may be open from within or outside, classifying it into internal or external open fracture respectively.

1) Internally open fracture end pierces the skin from within resulting in an open fracture
2) Externally open fracture causes fracture by lacerating the soft tissues and skin over the bone and breaks the bone causing an open fracture.

Exposure of the open fracture makes it more prone to infections.

BASED UPON THE COMPLEXITY OF TREATMENT

Simple fracture: It is a fracture in two pieces which is easy to treat

Complex fracture: It is a fracture in multiple pieces which is usually difficult to treat.

BASED UPON THE FORCE CAUSING FRACTURE

High velocity injury: These are sustained due to severe trauma force, as in case of road traffic accidents. There will be severe soft tissue injury in these type of fractures. The fracture ends will be devascularised. These are often unstable and take times to heal.

Low velocity injury: These are sustained due to mild trauma force, as in case of a fall. The soft tissue injury associated will be minimal. So these fractures have a good prognosis.

BASED ON THE PATTERN OF FRACTURE

Transverse fracture: The line of fracture is perpendicular to long axis of bone. It is caused by bending or tapping force.

Oblique fracture: The line of fracture will be oblique caused by a bending force which has a component along the long axis of the bone.

Spiral fracture: The line of fracture runs spirally in more than one plane, which is caused by a primarily twisting force.

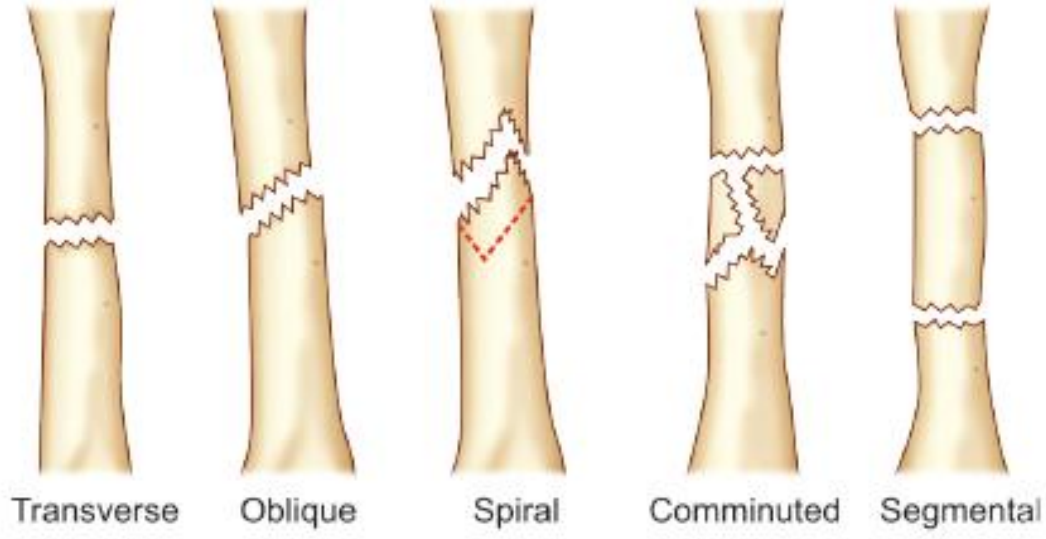


FIGURE V: FRACTURE PATTERNS

Comminuted Fracture: This fracture consists of multiple fragments which are caused by a compression or crushing force along the long axis of the bone.

Segmental Fracture: This type consists of two fractures in a single bone at different levels. This fracture will have a combination of two or more patterns.

AO CLASSIFICATION OF HUMERUS[7]

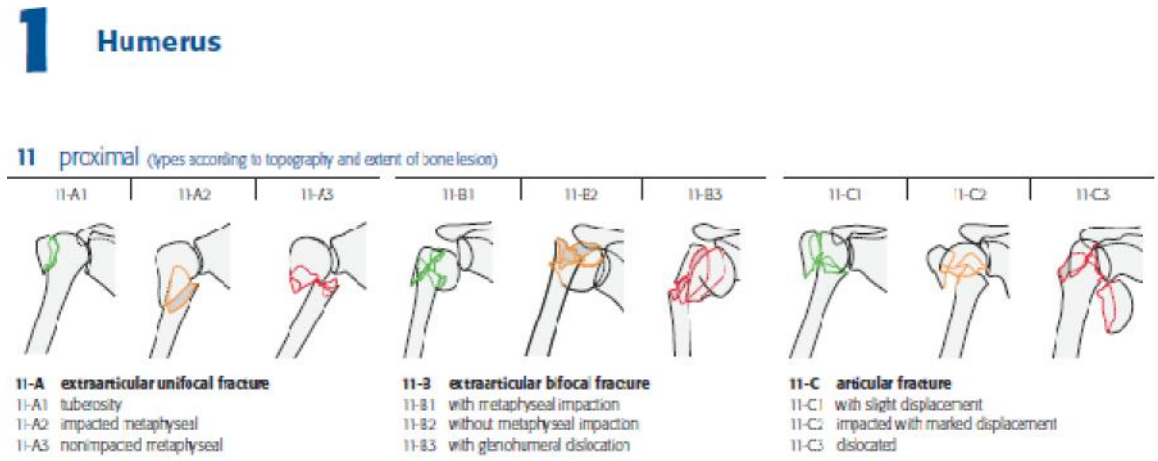


FIGURE VI: AO CLASSIFICATION OF PROXIMAL HUMERUS

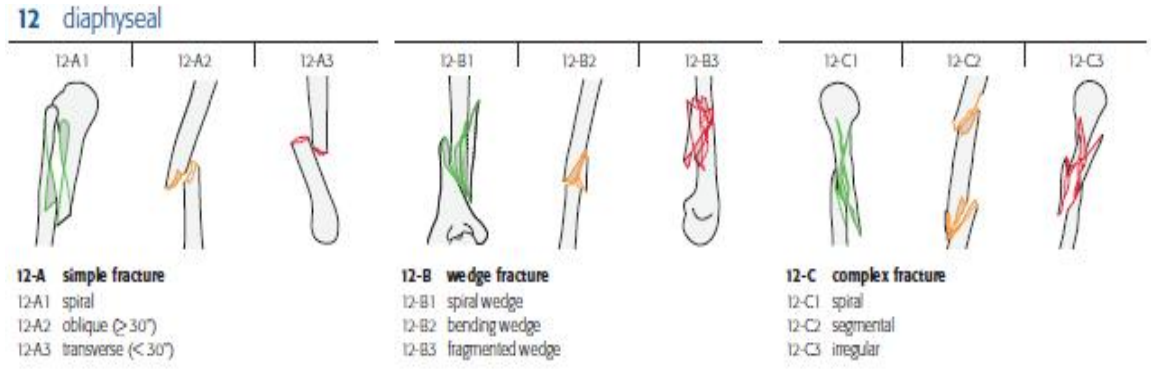


FIGURE VII: AO CLASSIFICATION OF DIAPHYSEAL HUMERUS

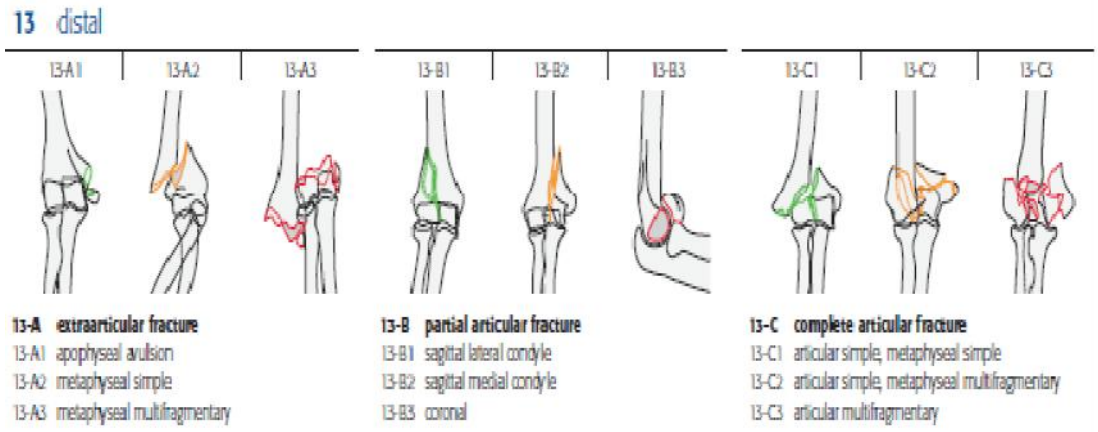
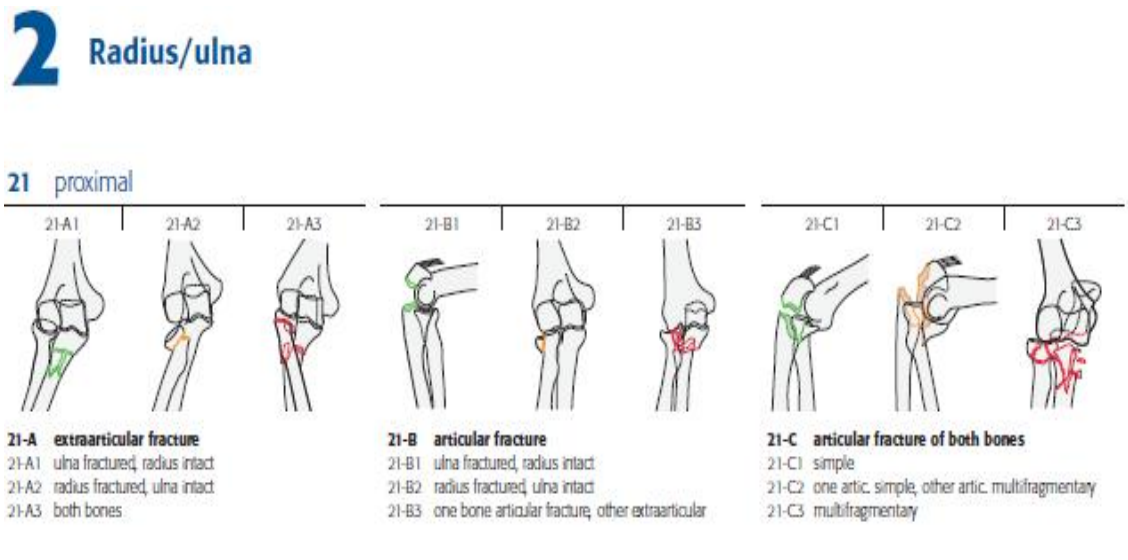
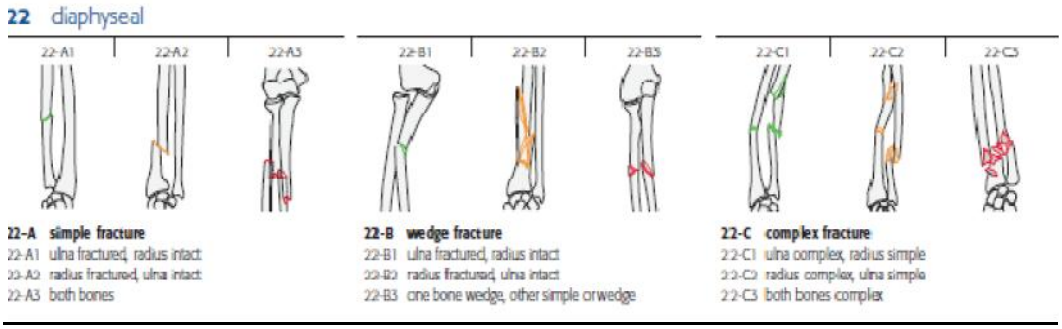


FIGURE VIII: AO CLASSIFICATION OF DISTAL HUMERUS





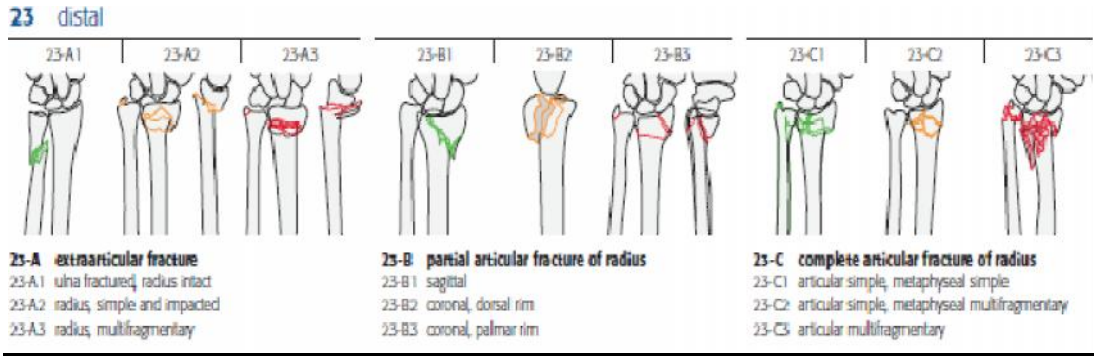


FIGURE IX: AO FRACTURE CLASSIFICATION OF RADIUS & ULNA[7]:

FRACTURE HEALING[5,11,12]

Fracture healing occurs in many ways similar to that of soft tissue wounds, the only exception being soft tissue heals with fibrous tissue whereas bone heals with mineralized mesenchymal tissue i.e., bone. There are various stages involved in fracture healing.

STAGES IN FRACTURE HEALING

- Stage of haematoma
- Stage of granulation tissue
- Stage of callus
- Stage of remodeling
- Stage of modelling

Stage of haematoma: Following a fracture, blood vessels gets torn leaking blood in the bone and forms a haematoma surrounding the fracture. The local soft tissues and the periosteum gets stripped off from the ends of the fracture resulting in ischaemic necrosis of fracture ends. Due to loss of blood supply some osteocytes die and others get sensitized to respond by differentiating into daughter cells(precursor cells) contributing in the healing process. This stage usually lasts upto 7 days.

Stage of granulation tissue : The sensitized daughter cells produce cells which gets differentiated to form blood vessels, fibroblasts, osteoblasts etc. They collectively form a soft granulation tissue in between the fracture fragments providing an anchor to the fracture. The blood clot forms a loose fibrous mesh which acts as a framework for the growth of fibroblasts and new capillaries. The clot gets removed by macrophages, giant cells and other cells in the granulation tissue. This is the stage where bone healing differs from that of soft tissues. In soft tissue, fibrous tissue replaces granulation tissue whereas in bone the granulation tissue differentiates to form osteoblast which forms bone. This stage lasts for 2-3 weeks.

Stage of callus: In this stage the granulation tissue differentiates into osteoblasts which forms an intercellular matrix which gets filled with calcium salts resulting in formation of the callus also known as woven bone. The callus is considered to be the first sign of union i.e., visible on X-rays. Callus is usually seen 3 weeks

after the fracture. Formation of this callus provides good strength to the fracture. Formation of callus is slower in adults than in children. It is slower in cortical bones than in cancellous bones. This stage lasts for 4-12 weeks.

Stage of remodeling: This stage was earlier known as stage of consolidation. In this stage the callus gets replaced by mature bone with a lamellar structure. This change occurs as a multicellular unit, where a pocket of callus gets replaced by a pocket of lamellar bone. This process lasts from 1-4 years.

Stage of modeling: This stage was earlier known as stage of remodeling. In this stage the bone gradually gets strengthened. The cortices gets shapened at the periosteal and endosteal surfaces. Local bone strains such as the weight bearing stress and muscle force stimulates this stage as soon as the person resumes activity. This stage is more predominant in children whereas it occurs to a limited extent in adults.

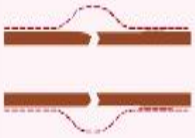
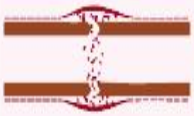
Stage of healing	*Approximate time*	*Essential features*
Stage of haematoma 	Less than 7 days	Fracture end necrosis occurs. Sensitisation of precursor cells.
Stage of granulation tissue 	Up to 2-3 weeks	Proliferation and differentiation of daughter cells into vessels, fibroblasts, osteoblasts etc. Fracture *still mobile.*
Stage of callus 	4-12 weeks	Mineralisation of granulation tissue. *Callus* radiologically visible. Fracture clinically united, *no more mobile.*
Stage of remodelling 	1-2 years	Lamellar bone formation by multicellular unit based remodelling of callus. Outline of callus becomes dense and sharply defined.
Stage of modelling 	Many years	Modelling of endosteal and periosteal surfaces so that the fracture site becomes indistinguishable from the parent bone.

FIGURE X: STAGES OF FRACTURE HEALING, FROST 1989

HEALING OF CANCELLOUS BONES

Bone healing in cancellous bone happens in a different pattern. Cancellous bone has no medullary cavity and is made of uniform spongy texture which creates an area of contact between the bony trabeculae. Bony union occurs between the trabeculae. Following haematoma and granulation tissue formation, mature osteoblasts forms callus in the intercellular matrix which unites the bony fragments.[4,5,11]

PRIMARY AND SECONDARY BONE HEALING

Primary fracture healing occurs when the fracture haematoma gets disturbed as in case of fractures that are treated surgically in case of open reduction. Bone healing occurs directly without callus formation which makes it difficult to evaluate the union in X rays.[11,12]

Secondary fracture healing occurs when the fracture haematoma is not disturbed. In case of secondary healing callus formation occurs which can be evaluated in X rays. Secondary healing occurs in case of fractures that are treated without disturbing the fracture haematoma like in case of closed reduction and conservatively treated patients.[11,12]

FACTORS AFFECTING FRACTURE HEALING[18,19]

- **Age of the patient:** Bony union in children occurs in half the time compared to that in adults.
- **Type of bone:** Cancellous and flat bones heal faster than cortical and tubular bones.
- **Fracture pattern:** Spiral fractures heal faster than oblique which in turn is faster than transverse fractures. Comminuted fractures occur due to high velocity trauma or in osteoporotic bones, heal slower.
- **Disturbance in the pathoanatomy:** Changes occur at the fracture site following a fracture which disturbs the healing process. Interposition of the soft tissue in between the fracture fragment is one such disturbance which delays fracture healing. Ischaemic fracture ends is another cause where the vascularity of the bone ends are cut off thus making the fracture union slower.
- **Type of reduction:** Proper apposition of the fracture helps in faster union of the fracture. Atleast half of the fracture surface has to be in contact for proper union in case of adults. Whereas in case of children, fracture union occurs even if there is only side to side contact of the bones.

- **Immobilization:** Immobilization of the fracture depends upon the site of fracture. Strict immobilization of fractures like neck of femur fractures has to be done to reduce pain and avoid further displacement of the fracture fragments. But there is no need for all the fractures to be immobilized (e.g: rib fractures, scapula etc.)
- **Open fractures:** Open fractures are more prone to go for delayed union as there are high chances of the bone to go for infection.
- **Compression at fracture site:** Compression at the fracture site enhances the bone union in cancellous bone whereas in case of cortical bones, fracture site compression enhances the rigidity of fixation leading to primary bone healing.

DIAMOND CONCEPT OF FRACTURE HEALING[20]

Bone is considered to be one of the organs that possess the potential for regeneration. Bone has considerable capacity of repair. Unlike other tissues bone is one of the few tissues that heal without fibrous scar formation. This unique characteristic of the bone helps in remodeling of the bone or in healing of bone fractures. The complex pathway of bone healing comprises of factors in molecular level at conjunction with biomechanical and physiological principles. Any deficit noted in the healing cycle will alter the physiological sequence and will affect the fracture healing leading to various complications. Properly timed and properly

aimed interventions are required to reverse the conditions that affect the healing cycle so that it progresses to union and increases the efficacy of orthopedic therapies.

The different stages of fracture healing renew the stages of endochondral bone formation. Bone healing comprises of primary and secondary healing. Primary healing refers to direct approach of cells in the bone cortex to re-establish the bone continuity that has been disrupted due to fracture. Primary healing requires absolute contact of the fractured fragments with complete stability. Secondary bone healing involves endochondral and intramembranous ossification leading to callus formation (2). Osteoprogenitor cells in the periosteum and mesenchymal stem cells get activated. Callus is the physiological reaction to the inter-fragmentary movements. It requires adequate blood flow and residual cell vitality.

In this cascade of events many local and systemic factors too play a key role. Multipotentmesenchymal stem cells are inducted in the fracture site or transferred to the fracture site in blood circulation. In response to a fracture bone marrow responds by reorganizing the cellular population of bone marrow to areas of low and high cellular density. In the areas of high cellular density the mesenchymal stem cells transform into cells with an osteoblastic nature.

The fracture hematoma is a source of signaling molecule for multiple growth factors (Vascular endothelial growth factor VEGF, Insulin like growth factor IGF, Platelet derived growth factor PDGF, Transforming growth factor β TGFβ, IL-1, IL-6, Fibroblast growth factor FGF, Tumor necrosis factor – a TNF-a) induce a cascade of events that helps in healing. These growth factors are secreted by monocytes, mesenchymal stem cells, macrophages, osteoblasts, osteocytes and chondrocytes. Various experimental studies have proven the efficacy of fracture healing by using growth factors.

The third important element that plays a key role in fracture healing is the extracellular matrix that acts as a natural scaffold. Various materials that have osteoconductive properties can be used as the bone scaffold. Usually osteoconductive materials along with osteogenic and osteoinductive properties are used in practice. Biomaterials like xenograft or allograft, polylactic acid, calcium based ceramics, bioactive glasses, hydroxyapatite, demineralized bone matrix and trabecular bone. These materials can also be combined with bone active growth factors in order to achieve maximum osteogenic effect.

Enhanced fracture healing is achieved by these three biological prerequisites. Various research and studies have been conducted to understand the complex of interactions between the osteogenic cells, osteoinductivestimulus and osteoconductive scaffolds are analysed in quest of optimal graft material.

However, there exists a fourth element which is quite mandatory for optimizing the bone fracture, i.e., mechanical stability. Mechanical stability acts as a crucial factor for bone healing and callus formation. Maturation of the callus depends upon the stability in the fracture site. Fracture fixation can be done by internal fixation or external fixation. Fracture healing depends upon the rigidity of the implant, absolute stability in the fracture, size of the fracture and interfragmentary strain. Relative stability, soft tissue envelope and vascularity surrounding the fracture site are considered to be essential. Fixation of the fracture site by splints, casts, external fixators, intramedullary nail and plates following open or closed reduction stabilizes the fracture site by reducing the interfragmentary gap and keeps the interfragmentary strain less than 10%. Fracture fixation along with bone grafting the mechanical material acts as an additional bone healing element which enhances the fracture healing and provides a stable mechanical environment to the fracture site resulting in a diamond shape concept of interaction.

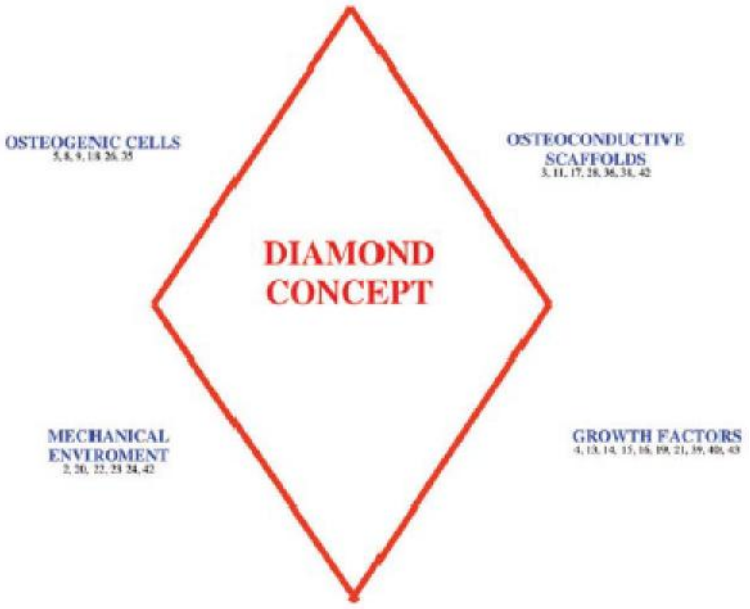


FIGURE XI : DIAMOND CONCEPT OF HEALING

PHYSIOLOGY OF BONE REPAIR

Bone tissue has got the capability to self-repair resulting in formation of new bone which has all the characteristics of a normal bone. Healing of the fracture or filling of the bone defect by an autologous cancellous bone graft is a result of interactions between osteoconductive matrix, osteogenic cells, cytokines and a stable environment with adequate blood supply. [13,14]

In certain rare cases, primary healing occurs in the cortices following a perfect fracture reduction and proper stabilization. Usually fracture healing comprises of enchondral and intra-membranous ossification. This complex process involves various events in four different stages: an inflammatory response,

formation of soft callus, formation of bony hard callus and bony union with remodeling. This involves a series of anabolic and catabolic events, some events are non-specific (production followed by remodeling of cartilaginous callus) and other specific events (formation of bony callus which later gets remodeled into normal bone). The end result of bone repair is the production, by cells of collagen matrix, the ossification of which restores the normal properties of bone. This histological stage of bone repair requires different cellular events like migration, proliferation and differentiation, the coordination of these are done by growth factors and cytokines.[13,14]

Inflammation plays an important role in the beginning of bone repair process. The release of Pro-inflammatory cytokines (interleukins IL-1β and IL-6, TNFα) is triggered by an injury. The chemotactic effects of these attract inflammatory cells, which results in stimulation of angiogenesis at the site of fracture. Cell proliferation and differentiation are regulated by molecular mechanisms which get elucidated partly. Till date there are no biological markers identified for monitoring of bone healing.[13,14]

CELLS INVOLVED IN BONE REPAIR[13,14]

Many cell types are mobilized by bone repair process. Even though the mobilized cells do not have a direct role in bone formation, the cells involved in

the angiogenic and inflammatory responses are essential in the bone formation mechanisms. These cells release cytokines and growth factors (PDGF, VEGF, Interleukins and BMP) which activate the mesenchymal stem cells (MSC) that are directly involved in bone repair.

Mesenchymal stem cells are the precursor of osteoprogenitor cells. MSC's are multipotent cells that can be produced in a reliable manner for clinical purposes. MSC does also play an important role in cell therapy for bone repair. Osteoclast lineage plays a major role in bone remodeling, but it is not used for clinical applications currently.

Friedenstein et al, was the first to describe formation of new bone from cultured bone marrow cells. Colony forming unit fibroblasts (CFU-F's) or fibroblast like cells are generated by bone marrow cells proliferated in vitro. Mesenchymal stem cells are multipotent non haematopoietic cells that have the capability of differentiating into functional cell types seen in different mesenchymatous tissue like muscle, tendon, adipose tissue, bone, cartilage and haematopoietic stroma.

The mesenchymal stem cells are identified in vitro based upon their ability to adhere to the plastic culture dishes and their ability to generate colony forming unit fibroblasts following several days of culture in a standard medium containing foetal calf serum. The mesenchymal stem cells gets differentiated into osteoblasts (bone tissue cells), chondrocytes (cartilage cells) and adipocytes (adipose tissue)

based on the available induction influences. There is no specific marker of MSC's available which makes their identification and extraction from the bone marrow cells difficult.

The mesenchymal stem cells were first identified in bone marrow, which was then identified in adipose tissue, cord blood, placenta, periosteum and other tissues. Stem cells from different sites have a similar phenotypic characteristic but they differ in their differentiation and proliferation properties. The stem cells can be identified only after culturing but native mesenchymal stem cells which are naturally seen in tissues are difficult to identify and have poor characteristics. These native mesenchymal stem cells have also been identified in the walls of blood vessels.

The source of mesenchymal stem cells that help in bone repair following a fracture is difficult to determine. In animal models, it has been identified to come from the periosteum, bone marrow and surrounding soft tissues. The most reliable source of mesenchymal stem cell is from the bone marrow, where about 0.001 % to 0.01% of all mononuclear cells in adults which decrease over age. 1 milliliter of bone marrow contains about $18+/-7x10^{6}$mononuclear cells inclusive of 612+/-134 mesenchymal stem cells. Bone marrow concentration and culturing techniques are valuable to expand the mesenchymal stem cell population available for clinical use.

There are various methods available now to expand the mesenchymal stem cells with current requirements for clinical use. The culture media that is used at present have no animal products and is based on human platelet lysates that are designed for optimal safety. In a time of 2-3weeks a 30ml of bone marrow sample collected from the iliac crest can generate several millions of mesenchymal stem cells depending upon the culture area. During the culture mesenchymal stem cell differentiation to cartilage, adipose or bone cells can be induced. Differentiation of the cultured mesenchymal stem cells to osteoblastic lineage (osteoinduction) is acquired by adding BMP or dexamethasone. Differentiation of the mesenchymal stem cells release cytokines and growth factors that regulate the bone repair process.

CELL THERAPY APPROACH TO BONE REPAIR[13,14]

Cell therapy is considered to be the best alternative of autologous bone grafting. Osteoprogenitor cells are implanted in large numbers at the injury site, either combined with matrix or alone. Bone marrow MSC's are the appropriate cells are considered to be the appropriate cells to induce bone repair. They are considered to have a very strong osteogenic potential and they can be easily obtained by culturing the aspirates from iliac crest. Several mesenchymal stem cell based cell therapies have been developed. These are developed with and without cell culturing and with or without matrix. Mononuclear cell fraction of the bone

marrow contains the mesenchymal stem cells which can be directly used by percutaneous injection of the aspirated bone marrow in the injury site. Bone marrow aspiration is done in heparin syringes. As large fractions of blood aspirations can dilute the bone marrow thus decreasing the mesenchymal stem cells, small fractions of 2-4ml of blood is aspirated. To increase the concentration of mesenchymal stem cells in the aspirate, the aspirated bone marrow can be centrifuged. Hernigou et al, after concentration by centrifugation obtained 2579 +/- 1121 mesenchymal stem cells per ml i.e., a 3-6 fold increase was obtained after aspiration. Before implantation of the mononuclear cells in the injury site, it can be combined with a synthetic or natural osteoconducting matrix (eg: coral or allogeneic bone graft).

In order to enable the expansion of mesenchymal stem cells the mononuclear cells can be cultured in vitro. This method has seen to generate millions of stem cells from only a few thousand that was present initially in the aspirate. Cultured mesenchymal stem cells can either be combined with biomaterials or injected percutaneously to form a construct i.e., appropriate for bone grafting.

In vitro culturing of the MSC's on biomaterials can be done for a few days or weeks. After obtaining biomaterial colonization and cell differentiation, the construct is directly implanted over the injury site. This is a procedure which follows bioreactor concept that is used in bone tissue engineering. One option

consists of implanting the construct in a muscle to promote angiogenesis, for few weeks. The muscle that contains the implant construct is directly transplanted and harvested into the injury site. An anastomosis is created with muscle vascular pedicle to ensure adequate blood supply. Thus turning the patient into his/her own bioreactor.

Depending upon the goal (mechanical strength or filling) and approach (percutaneous or surgical) various biomaterials are chosen to combine with the cells. Calcium phosphate ceramics in combination with hydroxyapatite and β tricalcium phosphate are more commonly used biomaterials. They are commonly available in granule form and rarely as stick which exhibits interconnected pores measuring 100-400 μm. These biomaterials help in proliferation, osteoblastic differentiation and adhesion of the mesenchymal stem cells. It also promotes the production of collagen matrix that undergoes mineralization. The biomaterials must facilitate the ingrowth of newly formed blood vessels from the surrounding tissues. Depending upon the biomechanical role played by the biomaterials, their absorption must be at a variable rate.

CELL THERAPY FOR BONE REPAIR[13,14]

Filling of bone defect or bone union is influenced by the size of the defect, quality of the surrounding skin and muscle, quality of blood supply and the site of defect.

Delayed union or nonunion without a bone defect : Delayed union and nonunion are one of the most common complications. The current standard of care for delayed union and nonunion are internal fixation combined with autologous cancellous bone grafting. In case of bone exposure muscle flap is used instead. The autologous bone graft supplies bone marrow cells, mesenchymal stem cells and bone matrix. It is also considered to be a form of cell therapy.

Initial attempts at cell therapy relied on bone marrow: Initially one method consisted of aspirating the bone marrow and injecting it percutaneously over the injury site. Connoly et al, in 1991 made a positive report in correlation between cell concentration in bone marrow aspirate and bone union. 18 of 20 patients with tibial nonunion were treated successfully. Similar studies were conducted and all were successful.

There was another option of increasing the number of injected mesenchymal stem cells by concentrating the bone marrow aspirate. In a study conducted by Hernigou and Beaujean, a decrease in mesenchymal stem cell density was noted at the nonunion site. Depending upon the mesenchymal stem

cell concentration the healing rate increased. Patients with delayed fracture union were noted to have received less than 1000 MSC's per ml and lesser than 30000 MSC's overall whereas patients for whom fracture have healed have received 1500 MSC's per ml and 54000 MSC's in a volume of 20ml.

Bone marrow aspirates of 300-500ml are required to find out the bone marrow concentration in cell therapy accredited laboratories. A small amount of the recovered mononuclear cells are kept in the laboratory to determine the colony forming unit fibroblasts count which is an indicator of the mesenchymal stem cell concentration in the reinjected mononuclear cells. Smaller bone marrow samples that are obtained intraoperatively have lesser concentration and produces very few mononuclear cells. The number of reinjected mesenchymal stem cells cannot be evaluated by colony forming unit fibroblast quantification. In procedures where MSC's is not required in large number, this procedure can be used.

Use of concentrated or unconcentrated bone marrow with a biomaterial has been assessed by few studies. It has been recognized as an option for day to day practice.

Mesenchymal stem cell population can be expanded in vitro: Culture expanded mesenchymal stem cells and platelet rich plasma were used in lengthening of 51 femurs or tibias showed good improvement in healing when compared with 60 control bones. Healing index was found to be better in femur than tibia, suggesting that blood supply and soft tissues too play a vital role. Cultured MSC's were used

on 64 closed long bone fractures and showed improved callus formation when compared with the control group.

Treatment of bone defects*:* Treating a bone defect is more challenging. The rate of success depends on the quality and size of the defect and the surrounding soft tissues. For a bone defect of size less than 3 cm only autologous bone graft is sufficient. Masquelet procedure is a useful technique to treat large bone defects, in this procedure, cement is implanted which induces the formation of a membrane. Autologous cancellous bone graft is also implanted along with the cement. The membrane which gets formed has osteo-inductive properties which promote the union of the cancellous bone graft. The risk of graft necrosis can be avoided by usage of vascularized bone grafts. Implantation of allografts in large defects has some disadvantages like re-inhabitation, risk of absorption and risk of spread of infection.

Quarto et al reported the use of cultured bone marrow mesenchymal stem cells and combined it intra-operatively with blocks of hydroxyapatite to fill large bone defects. 3 patients with defects in tibia, humerus and ulna were treated successfully. Healing of the defects were confirmed after 6-7years in a subsequent study.

Next generation Mesenchymal stem cell culture involves culturing of MSC's in a biomaterial for several days or weeks and then implanted into the defect. This technique was followed by Morishita et al, and was successfully

implanted in 3 patients with benign bone tumour. Bone marrow MSC's were proliferated for 2 weeks and were allowed to culture on hydroxyapatite granules for 2 weeks. Culturing of MSC's was done in a medium which was designed to promote osteoblastic differentiation. This construct was implanted into bone defects. Osteo-integration was found to be satisfactory after 29 months. Using this technique Meijer et al, conducted a study with positive outcomes in 6 patients. This construct was implanted in jaw defects. 4 months post-operative biopsies were obtained which showed bone formation in 3 patients.

SAFETY REGULATIONS AND REQUIREMENTS

In present situation only autologous mesenchymal stem cells are used for cell therapy. Intra-operatively using small centrifuges, bone marrow aspirate can be concentrated, this does not require any authorization but it is to be performed under the responsibility of the surgeon. But if the sample is taken out of the facility for concentration of bone marrow then the facility requires an accreditation. Different policies are followed by different countries in cell therapies.[17]

FUTURE IN CELL THERAPY

Osteonecrosis, delayed union and non-union will be the main indications for usage of cell therapy in bone repair. Multiple trials have been conducted on usage of the bone marrow cells in combination with biphasic calcium phosphate granules in patients with non-union or delayed union. Trials have been conducted on injection of mesenchymal stem cells in patients with avascular necrosis of femoral head. Hydrogel based solutions which will deliver biomaterials and cells percutaneously can be expected in the near future.[17]

PLATELET CONCENTRATES [21]

Bioactive surgical additives regulate inflammation and increase healing process. Development of such bioactive surgical additives is one of the great challenges faced in clinical research at present. Hard and soft tissue healing involves both intra and extracellular events which are regulated by signaling proteins. It has been found that platelets not only play a crucial role in hemostasis, but also in wound healing. Platelets are derived from bone marrow megakaryocytes, measuring 2-3 µm in diameter. They are anucleate cytoplasmic fragments containing many granules, 2 prominent membrane structures, few mitochondria, dense tubular system and surface connected canalicular system. The α granules are oval or spherical structures with size ranging from 200-500 nm in

diameter, each α granules are enclosed by a unit membrane. These consist of proteins that play a vital role in wound healing namely insulin like growth factor (IGF – 1), platelet derived growth factor (PDGF) and transforming growth factor (TGF-β). After activation, platelet cell membrane fuses with α granule. Some of the secretory proteins get transformed to a bioactive state. The active proteins then get secreted which binds to the transmembrane receptors of the target cells. Intracellular signal proteins get activated as soon as they bind to the target cells resulting in expression of a gene sequence which directs collagen synthesis, cellular proliferation, osteoid production etc.

Platelet growth factors are a good source of cytokines that help in healing which can be used for clinical applications. Various techniques of autologous platelet concentrates were developed and were applied in oral and maxillofacial surgeries which lead to a fibrin and platelet concentrate development for topical applications. Blood was collected with anticoagulant and centrifuged to form a base of platelets with a layer of acellular plasma and red blood cells. The base of platelets again was placed in a plasma solution rich in fibrinogen and was then injected on the surgical site, mostly in the presence of calcium and bovine thrombin.

FIBRIN SEALANTS

Fibrin sealants were the first surgical additives to be used which was available from late 1970's in Europe. Fibrin sealants, fibrin tissue adhesives or fibrin glues are a human plasma derivative that imitates the final stage of blood coagulation, which forms a fibrin clot. These were used as melting agents of particulate bone substitutes, tissue sealing and topical hemostasis. Risk of cross infection in commercial adhesives led to development of fibrin sealants from the patient's own plasma. Types of fibrin sealants available are:

1. **Homologous Fibrin sealant:** It is available as freeze dried two component preparations namely Fibrinogen/fibronectin/factor XIII concentrate which is dissolved in an antifibrotic solution (aprotinin usually) and Thrombin concentrate dissolved in dilute calcium chloride. Mixing of these 2 components imitate the last stage of coagulation cascade which results in formation of a fibrin clot independent of the coagulation pathway.
2. **Autologous Fibrin sealant:** Fibrin sealants were prepared from patient's own plasma to avoid risk of transmitting infection. Bovine thrombin is used to initiate fibrin polymerization.

Fibrin sealants were mainly used in oral maxillofacial surgeries. They were used to treat:

- Alveolar ridge augmentation
- Treatment of recession
- Treatment of intrabony defects
- Sinus floor augmentation
- Bone regeneration involving dental implants
- Sinus floor augmentation
- To treat extraction wounds

Usage of fibrin sealants had their own limitations like

- Characteristics and composition of the sealants varies in homologous and autologous
- Autologous fibrin sealants are weaker and had lower resistance to stress than homologous sealants
- Beneficial effects of fibrin sealants for soft tissues were well documented whereas its role in orthopaedic surgery and periodontal surgery are still controversial
- Fibrin glue production was very expensive
- Fibrin glue had high risk of viral transmission

PLATELET RICH PLASMA – 1ST GENERATION PLATELET CONCENTRATE[21]

PRP (Platelet rich plasma), PC (Platelet concentrates) and platelet gels are autologous products with high platelet concentration. They had properties of fibrin sealants and they were combined with growth factors which provided delivery of the growth factor at the site of injury. The scientific fact that lies behind the use of these preparations is the fact that growth factors play a very crucial role in hard and soft tissue repair mechanisms. The growth factors have both chemotactic and mitogenic properties which promote the cellular functions that are involved in tissue healing, cell proliferation and regeneration.

TECHNIQUE

- Venous blood is collected with anticoagulant, in order to avoid the activation of platelets and degranulation
- The blood is first centrifuged (soft spin) at a speed of 1500 rpm for 5 mins allowing it to separate into 3 distinct layers
- The platelet poor plasma (PPP), some red blood corpuscles and platelet rich plasma (PRP) are formed after the first centrifugation. The platelet rich plasma is carefully aspirated using a sterile syringe and is transferred to a tube which does not contain anticoagulant.

- The second tube is centrifuged at a speed of 6300 rpm for 20 mins which is longer and faster when compared with the first centrifugation process (hard spin). This allows the platelets to get concentrated in the bottom of the tube and it subsequently forms 3 distinct layers again
- Following the second centrifugation it becomes easy to collect the PRP. It is collected using a syringe. The platelet poor plasma can be discarded and the concentrated platelets along with serum can be used as a suspension. The tube is then gently shaken to get a ready to use cPRP (concentrated platelet rich plasma)
- In order to make the gelling of platelet concentrate quicker, the cPRP is mixed with calcium chloride and bovine thrombin using a mixing syringe. During the cPRP preparation fibrinogen is also concentrated with it. Polymerization of this will result in formation of a fibrin matrix with adhesive and hemostatic properties.

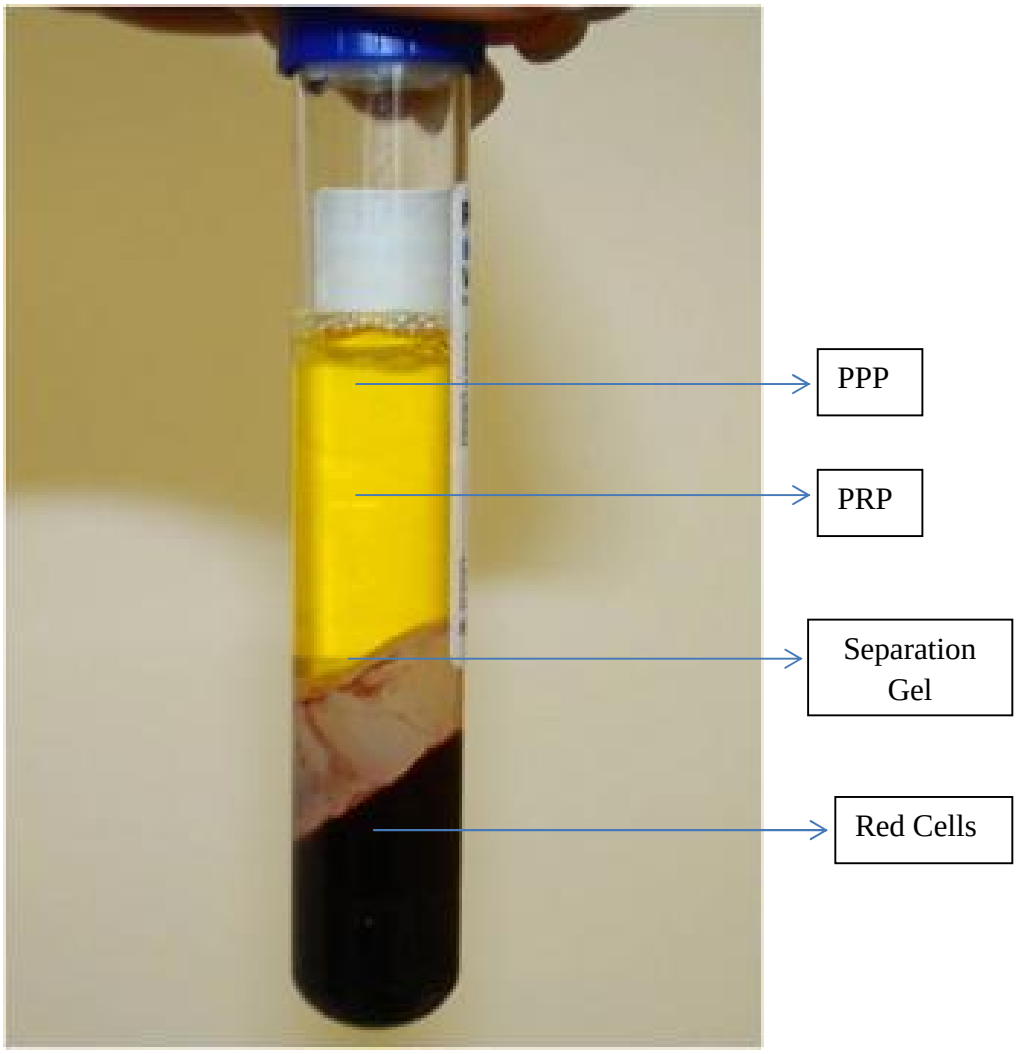


FIGURE XII: PRP WITH 3 DISTINCT

CLINICAL APPLICATIONS

- Used in sinus lift procedures
- Socket preservation
- Ridge augmentation
- Oral/nasal fistula repair
- Alveolar cleft palate repair
- Jaw reconstruction surgery
- Intra bony defects
- In soft tissue procedures

PLATELETS AND FIBRIN[23]

Being the second numerous corpuscles in the blood, Platelets lack megakaryocytes in the cytoplasmic fragments of 7 to 10 days lifetime and normal peripheral blood concentration is 150-450 x 10(9)/L. The unactivated platelets are biconvex discoid shaped measuring 2.0-4.0 by 0.5 µm approximately and a mean volume of 7-11 fl. The Peripheral area of Platelets constitutes a phospholipid membrane, microtublues series and canalicular system bridges the surface to cytoplasm. Mitochondria, lysosomes, peroxisomes, glycogen granules, alpha and dense granules can be identified in the the cytoplasm. Alpha granules (large macromolecules) constitutes about 15% in the total platelet volume containing the

platelet-specific and non platelet specific proteins ie., fibrinogen, fibronectin, thrombospondin and growth factors etc., Dense granules contains rich content of calcium, ADP, ATP, Serotonin and secondly the inorganic phosphorus. The leukocytes and growth factors are responsible for the healing and repair processes and the differentiation, proliferation, migration, cell metabolism with the participation of Polypeptides. In the site of Injury, Growth factors help in stimulation and attraction of stem cells which leads to the promotion of cell mitosis with induction of angiogenesis and osteogenesis.

The growth factors are trapped in the fibrin matrix after the platelet activation with the demonstration of mitogenic response stimulation of periosteum cells to achieve bone healing. Modulation of Platelet activation, proliferation and differentiation of leukocytes are carried out by the cytokines released from platelets playing a vital role in immunology and in mechanising inflammation.

Fibrin allows matrix for fibroblasts migration and endothelial cells involved in angiogenesis and healing of new tissues. The thrombin and factor XIIIa helps in the formation of fibrin network responsible for transformation of soluble fibrinogen –a large glycoprotein into a insoluble fibrin in three phases ie., proteolysis of fibrinogen by thrombin, polymerisation of fibrin monomers and fibrin stabilisation by factor XIIIa.

Due to physiological situations fibrin may suffer multiple variations such as the concentrations of calcium ions and fibrinogen. Co morbid conditions like diabetes and nephrotic syndrome may affect fibrin structure.

PLATELET-RICH FIBRIN[23]

An autologous leukocyte-platelet-rich fibrin matrix composed of a tetra molecular structure with cytokines, platelets, cytokines and stem cells within it which acts as a biodegradable scaffold that is favorable for the micro-vascularisation and help in migration of epithelial cells to its surface. It may also serve as transport medium in tissue regeneration and sustained release of growth factors in a time period of 1 and 4 weeks in wound healing.

Platelet Rich Fibrin acts as a biocatalyst in bone and soft tissue regeneration with inflammatory reactions. It may be used in bone grafts, promoting hemostasis, bone growth, maturation. Antibodies and Immunological properties may lead to leukocyte degranulation and enhance angiogenesis and anti-inflammatory reactions. PRF serves as a resorbable membrane for bone regeneration and helps in prevention of migration of non-desirable cells into bone defect which in turn provides immigration of osteogenic cells and allows underlying blood clot to mineralize. Naturally PRF normal membrane has paced degradability in 1-2 weeks and if fibers cross-linked, it prevents enzyme

degradation which leads to stable healing. In vitro research using mice by Kawase et al, suggests heat compression of PRF membrane with an indication of bone regeneration with this less cytotoxic technique by reducing the porosity and surface area which delays the degradation until 4 weeks. PRF membrane used in treatment of periodontal intrabony defects with protection of open wounds when suturing mucosal margins in open environment that cannot be easily done which enhances hard and soft tissue healing. In some trials and study, PRF membrane is used as graft in maxillary sinus augmentation. Undetected sinus membrane perforation sealed with using PRF membrane in Tofler et al, study in the lateral window osteotomy in lift procedure of maxillary sinus.

Platlet Rich Fibrin acts a biological connector which attracts the stem cell and favours the osteoprogenitor cells migration to the graft centre with neo-angiogenesis helping in surgical site wound healing.

Choukroun et al, studied using PRF membrane to see the potency of Freeze Dried Bone Allograft (FDBA) in maxillary sinus procedure enhancing the bone regeneration but the results showed slow healing time than implant. In histological studies, the healing time decreased from 8 months to 4 months. In addition of PRF to the bone graft can results in volume reduction of bone substitute and improve revascularisation by angiogenesis. Simonpieri et al, suggest that in bone defects, PRF with bone grafts gives better results.[23]

Yilmaz et al, compared the healing effects of β-TCP and PRF histologically and stereologically alone and in combination, in standardized bone defects in pig's tibia. In usage of both, significantly greater results were notified and PRF sometimes as a bioadhesive, facilitates the bone grafts manipulation. [23]

PROTOCOL FOR PRF PREPARATION

Venous blood sample is collected in 10ml tubes without anticoagulant. The tube is centrifuged at 3,000rpm for 10 minutes time. The resultant products will be of 3 layers, are:

1. Outermost top layer consists of acellular plasma
2. Middle layer of PRF clot
3. Red corpuscles at the bottom.

FIGURE XIII: 3 LAYERS OBTAINED AFTER CENTRIFUGATION

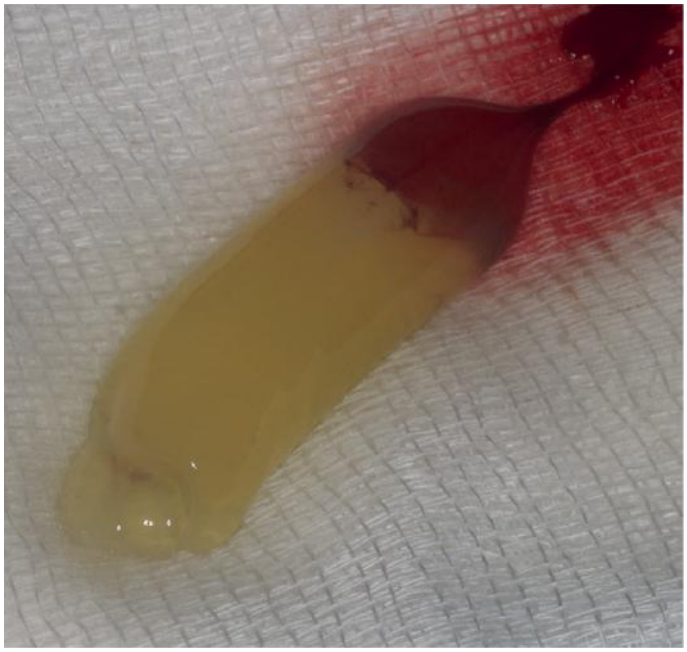


FIGURE XIV: PRF CLOT SEPARATED FROM RED CORPUSCLES BY TWEEZERS

After the formation of three layers, PRF clot necessarily must be kept in a sterile cup for 10 minutes approximately to allow the serum concentration release. Mazor et al, stated that the transformation of membrane will takes place in compression between the two sterile gauzes or in a specific tool.

In the beginning, fibrinogen is concentrated till the thrombin converts into fibrin in the upper segment of the tube. When the blood comes in contact to the glass surface from the top, it begins to coagulate due to lack of an anticoagulant. Silica surface is needed to activate the process of clot polymerisation. Due to this, PRF may be processed only in the dry glass tubes or glass coated plastic tubes.

Actually, the silica particles do not risk in cytotoxicity compared with like in PRP preparation bovine thrombin used.

As a net result, Platelets will be trapped in large proportion in the fibrin meshes. Successful preparation of PRF comprises of rapid blood collection and centrifugation immediately earlier to the initiation of clotting.

MERITS IN PLASMA RICH FIBRIN

- Simple preparation and efficient technique with one step centrifugation with easy accessibility obtained by an autologous blood sample.
- Minimal blood manipulation and addition of thrombin is not required externally because polymerisation is an out and out natural process without any immunological reactions.
- Naturally fibrin with growth factors has a relatively longer period of time and tissue generation.
- Can be used alone or in combination with bone grafts and enhance the bone grafts healing rate.
- It’s cost effective and rapid when compared with recombinant growth factors used in bone grafts conjunction.

- Donor site surgical procedure can be avoided which results in patient discomfortability during the early wound healing phase.
- PRF studies are more efficient and less controversial.

DEMERITS OF PLASMA RICH FIBRIN

- Being an autologous blood process, the final outcome and success rate will be less. Directly in its quick handling and in relation to blood collection and transference in centrifugation.
- Glass coated tube is must to get clot polymerisation
- Possible refusal of treatment by the puncture required for blood collection.

OTHER CLINICAL USES

- Oral surgeries i.e., In periodontal bone defects: achieving a probing depth reduction and a defect fills in radiography
- 90% Osteitis was identified in surgical sites of the 3rd molar in localised osteitis and as a supplementation to palatal wound healing after a gingival graft implantation.
- It can be used as a scaffold in pulp revascularisation procedures of tooth necrosis as it is high in growth factors and increase the cellular proliferation, angiogenesis augmentation, differentiation and in turn

acts as a matrix for tissue growth and regulation of inflammatory reaction.

- Alveolar ridge height preservation in multiple extractions and in alveolar defect used as in bone regeneration after implantation.
- Reconstruction of large bone defects, post cancer surgery and to fill cavities in plastic surgeries, also with an adipocyte graft in a lipostructure.
- PRF as in membrane form, can be used in otologic surgery.

BIPHASIC CALCIUM PHOSPHATE (BCP)[24]

Different bone graft materials has been developed and used for reconstruction in orthopaedics. The autogeneous bone graft is considered to be the gold standard due to its superior capacity in bone formation. One of the main clinical drawbacks is morbidity of the donor site and uncontrolled resorption rate. [24]

Biphasic calcium phosphate is an alloplastic grafting product which is synthetic in origin. It is osteoconductive and is biocompatible. It releases controlled levels of calcium ions over time which favors the formation of an apatite layer. The bioactivity displayed by BCP is due to the apatite layer. The bioactivity displayed by BCP is responsible for its osteoconductivity and osteoinductivity. [24]

The biomaterial surface supports growth of mature osteoblasts and direct apposition of the bone to the surface in case of osteoconduction. Whereas in case of osteoinduction, it favors the recruitment of undifferentiated or immature cells and stimulates its differentiation to the osteoblastic lineage which stimulates the osteogenesis.

Ca-Ps and hydroxyapatite are the grafting materials available which is very stable and maintains space effectively. But these have low osteoconductivity. In contrast, β tricalcium phosphate is rapidly replaced by new bone and is more biodegradable.

Biphasic calcium phosphate which is composed of hydroxyapatite and β tricaclium phosphate was developed to overcome the disadvantages of each materials and there are many studies which demonstrates that BCP can be used as bone substitutes successfully.

The combination of BCP with PRF opens the door to therapeutic alternatives and it also improves the pre existing ones. It increases the versability of the bone substitute materials. PRF holds the particles of the BCP together acting as a biological adhesive. Thus helping its manipulation easier and it also provides the survival and vascularization of the graft.[24,26]

LITERATURE FROM OTHER ARTICLES

Abdelgmagid et al (2015), conducted an experimental animal study in Egypt on Comparison between the use of PRF with and without BCP for osseo integration around dental implants. The study was conducted over eight mongrel dogs. Among the 8 mongrel dogs 4 were taken as cases and 4 were taken as controls. This study concluded that PRF in combination with BCP favored the formation of new bone.[24]

A histological and histomorphometric study was done by Zhang et al, in 2011 on Effects of Choukroun's PRF on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation. Among the 11 patients taken 6 patients were in the test group (Bio-Oss and PRF) and 5 were in the control group and was followed up for 6 months. The study concluded that percentage of new bone formation in the PRF group was about 1.4 times higher than in control group.[9]

In 2013,Ozdemir et al, conducted an animal histomorphometric study in New Zealand on Effects of PRF alone used with rigid titanium barrier. The study included 24 male adult rabbits which were divided into 4 groups (Experimental: Control – 3:1) and was followed up for 3 months. This study concluded that PRF

alone group showed more new bone formation when compared with the control group.[8]

In 2013, Bolukbsi et al, in Turkey conducted a histologic and histomorphometric study on the use of PRF in combination with BCP in treatment of bone defects. 6 sheeps were used in this study and were divided into 4 groups. The defects created in the sheep's tibia were grafted with BCP, PRF, BCP + PRF and a control group. They were evaluated during 10, 20 and 40 days. The study revealed that there was a histomorphometric increase in bone formation with addition of PRF + BCP.[3]

Tatullo et al in 2012, conducted a clinical and histological study in Italy on PRF in reconstructive surgery of atrophied maxillary bones. 60 patients participated in this study. The study concluded that the use of PRF and piezosurgery decreased the healing time from 150 days described in literature to 106 days.[10]

MATERIALS AND METHODS

This study was done to find out PRF+BCP's effectiveness in fracture healing and to compare its efficacy in early callus formation which was compared with a group of controls.

SOURCE OF STUDY

PSG Institute of Medical Sciences and Research, Coimbatore

STUDY DESIGN

It was a prospective randomized open control trial.

STUDY DURATION

The study was conducted for a period of 2 years from 2015-2017.

INCLUSION CRITERIA

- Patient's aged between 20-70 years
- Patient's acceptance
- Upper limb diaphyseal fractures treated by plate osteosynthesis
- Closed fractures

EXCLUSION CRITERIA

- Severe comminuted fractures
- Fractures with bone grafting
- Infected fractures
- Smokers
- Alcoholic
- Diabetic patients
- Open fractures
- Pathological fractures.

SAMPLE SIZE DETERMINATION

- A total of 26 patients in two groups.
- 13 patients in each group.

METHOD OF DATA COLLECTION

The study was conducted on patients with diaphyseal upper limb fractures who were treated as in-patient in PSG Institute of Medical Sciences & Research in Coimbatore.

A total number of 26 patients with upper limb diaphyseal fractures participated in this study. All the patients who participated in the study satisfied the inclusion and exclusion criteria.

Informed consents were obtained from the patients. Detailed clinical history and other relevant data were collected from the patient. For each and every patient the following details were entered: name, age, sex, in-patient number, out-patient number, type of fracture and co-morbid conditions.

The study was done to compare the efficacy of platelet rich fibrin in combination with biphasic calcium phosphate in fracture healing.

This was compared with a group of controls. It was evaluated by taking X-rays postoperatively to assess the fracture healing. X rays were taken every month from the date of surgery.

This study is divided into two groups: Study group and Control group

The Study group patients with upper limb diaphyseal fractures underwent open reduction and internal fixation with application of PRF + BCP over the fracture site.

The Control group consisted of patients with upper limb diaphyseal fractures undergo current standard of care i.e., open reduction and internal fixation without usage of PRF + BCP.

Sample size in each group

Study Group - 13 patients

Control Group – 13 patients

PREPARATION OF PRF (PLATELET RICH FIBRIN)

Based upon the studies by Choukroun et al, PRF was prepared. Venous blood of 30ml was collected from the patient in sterile vacutainers without any anticoagulant. These tubes were immediately centrifuged at a speed of 3000 rpm (revolutions per minute) for 15minutes. After centrifugation 3 layers were obtained, acellular plasma (platelet poor plasma) were concentrated at the top; fibrin clots and red corpuscles which got concentrated at the bottom of the test tube and was removed from the tube with a scalpel. PRF clot was immediately separated from red corpuscles by tweezers. This clot was cut into small pieces and mixed with graft material, BCP.

INTRAOPERATIVE PROCEDURE

Patients in Study Group, received PRF + BCP along with open reduction and internal fixation. PRF was prepared intraoperatively by collecting 30ml of venous blood and centrifuged immediately intraoperatively. PRF clot separated from the red corpuscles was cut into pieces and mixed with bone graft substitute BCP. PRF was mixed with BCP to form a paste like material. Following open reduction the fracture was fixed using a plate. The PRF + BCP that were prepared will be applied directly over the fracture site following the plate fixation and the surgical wound was closed in layers.

Patients in Control Group consisted of 13 patients who were controls. Open reduction and plate fixation was done. These patients did not receive PRF + BCP. These patients received current standard of care.

PRF AND BCP IN SEPARATE BOWLS

FIGURE XV: PRF BEING SEPARATED FROM THE RED CORPUSCLES

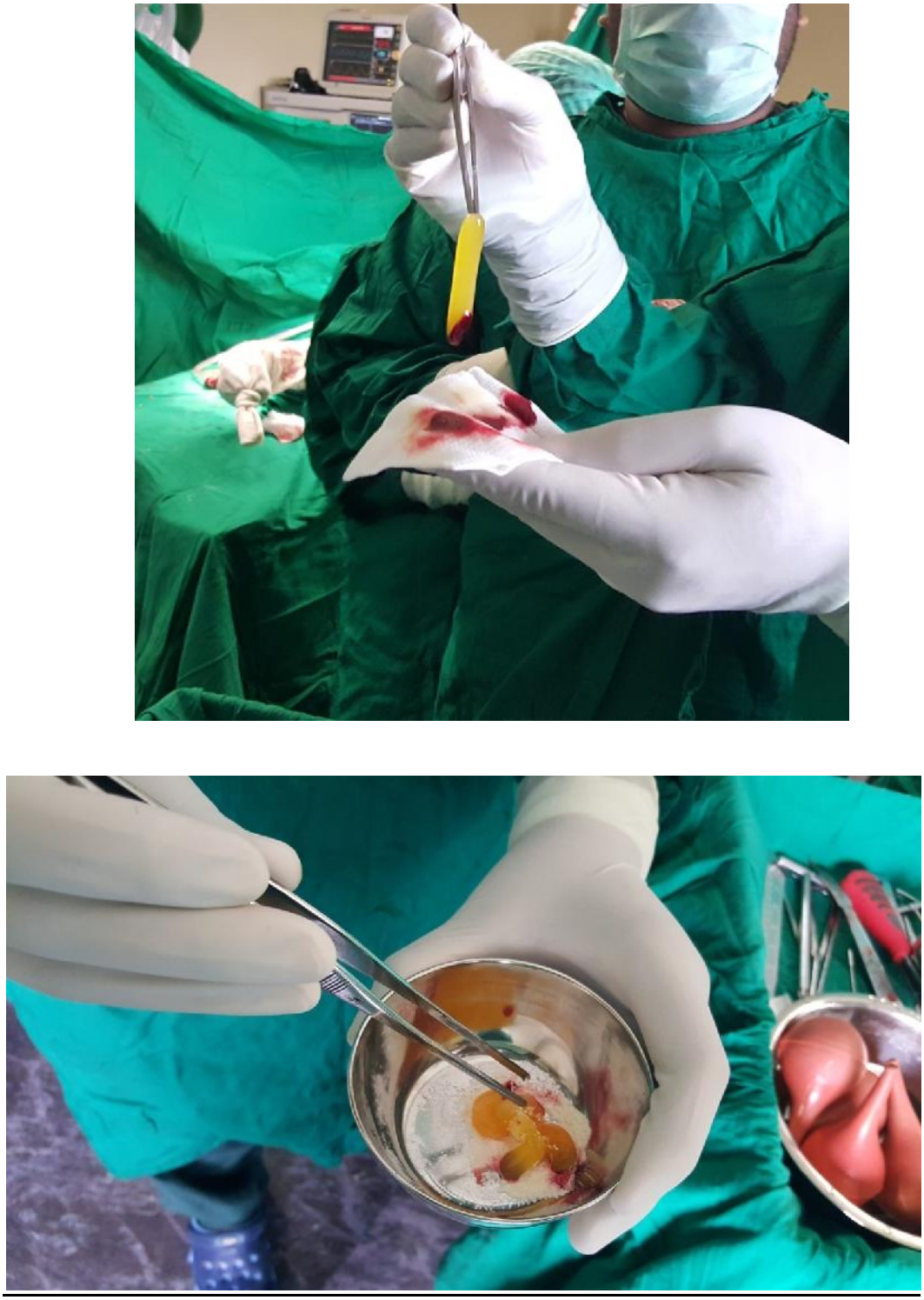
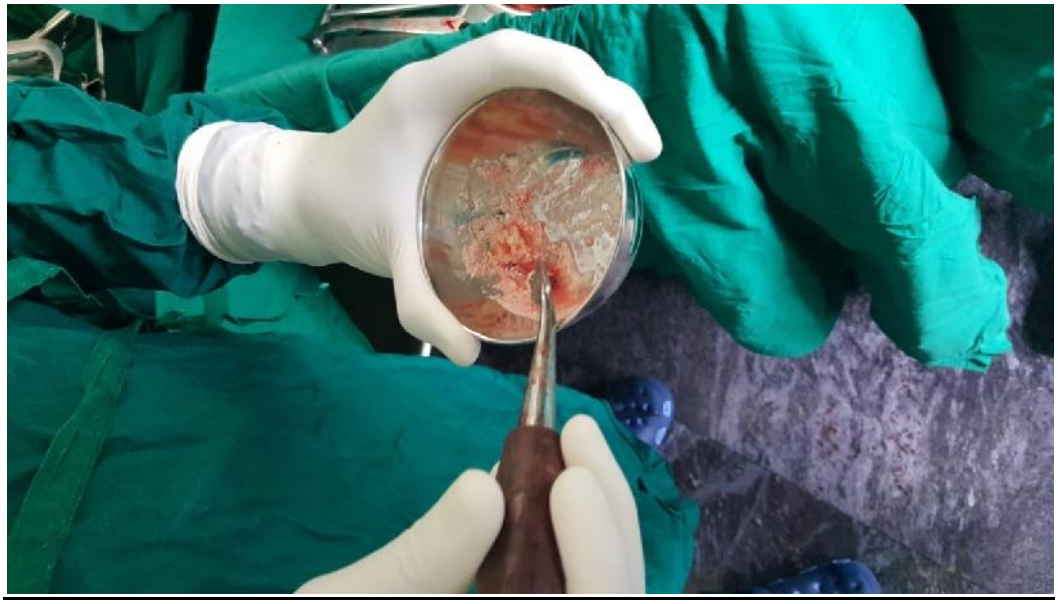


FIGURE XVI: PRF CUT INTO SMALL PIECES AND MIXED WITH BCP



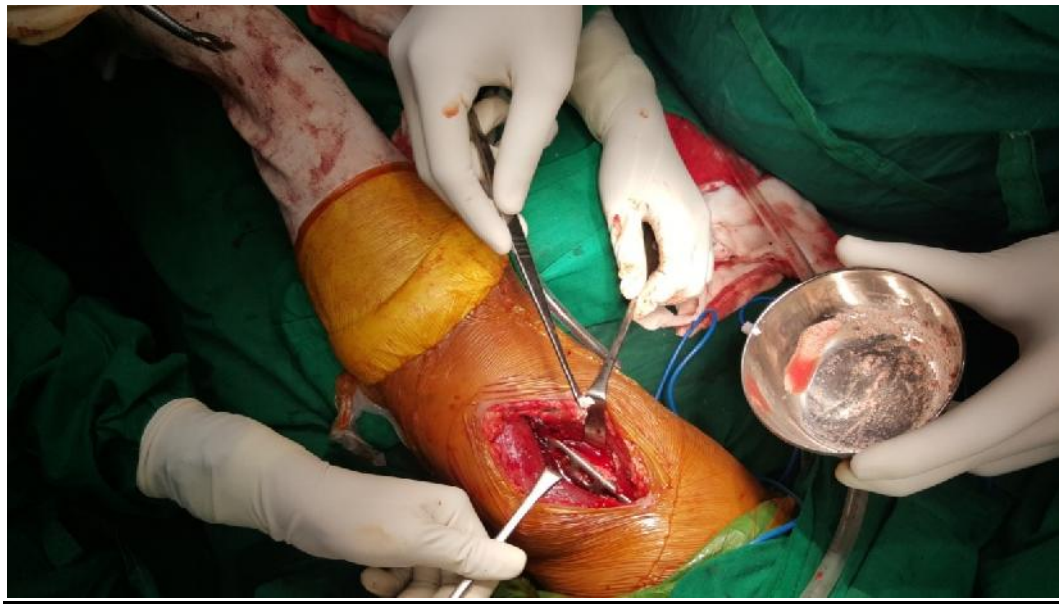


FIGURE XVII: PRF + BCP AUGMENTATION OVER THE FRACTURE SITE

POST-OPERATIVE FOLLOW-UP

Post operatively fracture healing was assessed using X rays. The aim of the study was to assess the rate of fracture healing and to test the efficacy of PRF + BCP in a period of 5 months. X rays were taken every month and the time taken for appearance of callus in the X rays were considered to be the beginning of fracture healing process. If the healing does not occur till 5 months, it will be considered to be non-union. Each X ray comprises of AP (Anteroposterior) and Lateral views. X rays of the operated limb were taken and were used to assess the fracture healing. In reference with Radiological Union Score (RUS) system fracture was assessed. Time taken for the appearance of the callus was noted in both AP and Lateral views were notedand compared.

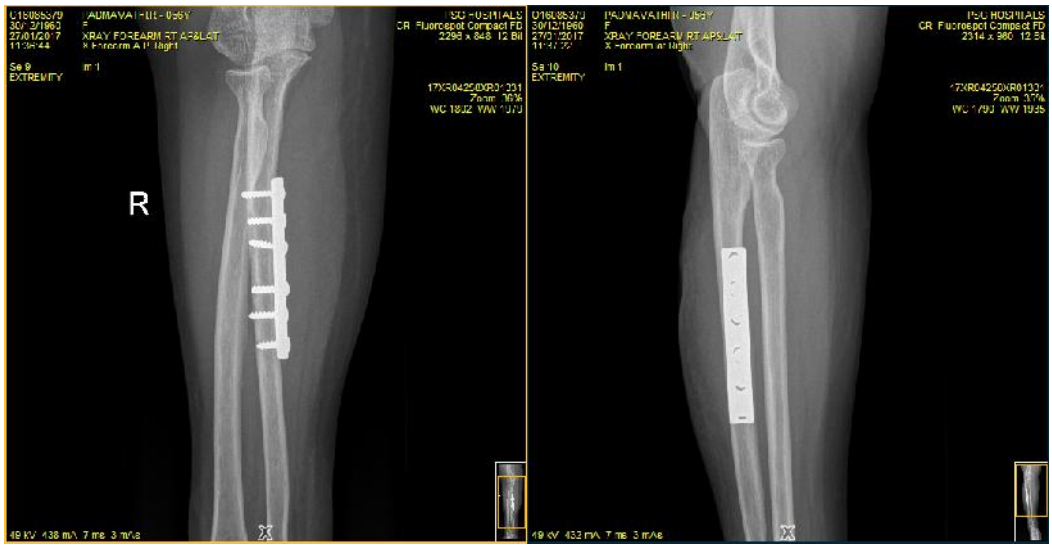


FIGURE XVIII: PICTURE OF AN X RAY SHOWING FULLY FORMED CALLUS.

STATISTICAL ANALYSIS

The data was compiled and analysed by using IBM SPSS Statistics 21.0 trial version. Descriptive Statistics like Mean, Standard deviation and Inferential statistics such as Chi-square test and Fisher's exact test were used for the analysis of the data. A p value of <0.05 was considered as significant.

RESULTS

Table 1: Profile of the subjects taken in PSGIMSR, Coimbatore		
PARAMETERS	**FREQUENCY (n = 26)**	**PERCENTAGE**
GENDER		
MALE	16	61.5%
FEMALE	10	38.5%
AGE (in years)		
20 – 34	11	42.3%
35 – 49	9	34.6%
> 50	6	23.1%

Table 1 represents, among the total study subjects (26) participated, it comprised of 16 males (61.5%) and 10 females (38.5%). The overall mean age & standard deviation was 38.69±13.97 years.

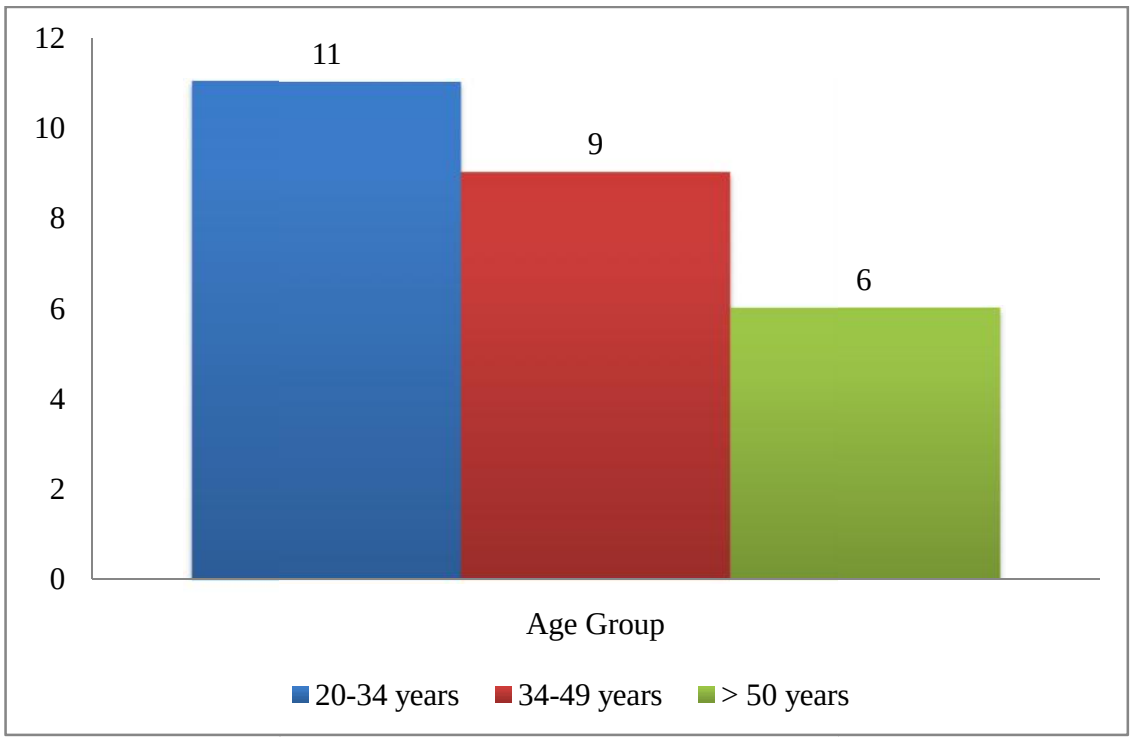


FIGURE XIX: BAR DIAGRAM REPRESENTING AGE –WISE DISTRIBUTION AMONG THE STUDY SUBJECTS (n=26)

Figure I depicts age-wise distribution among the study subjects. Most of the subjects (42.3%) were in the age group of 20 to 34 years.

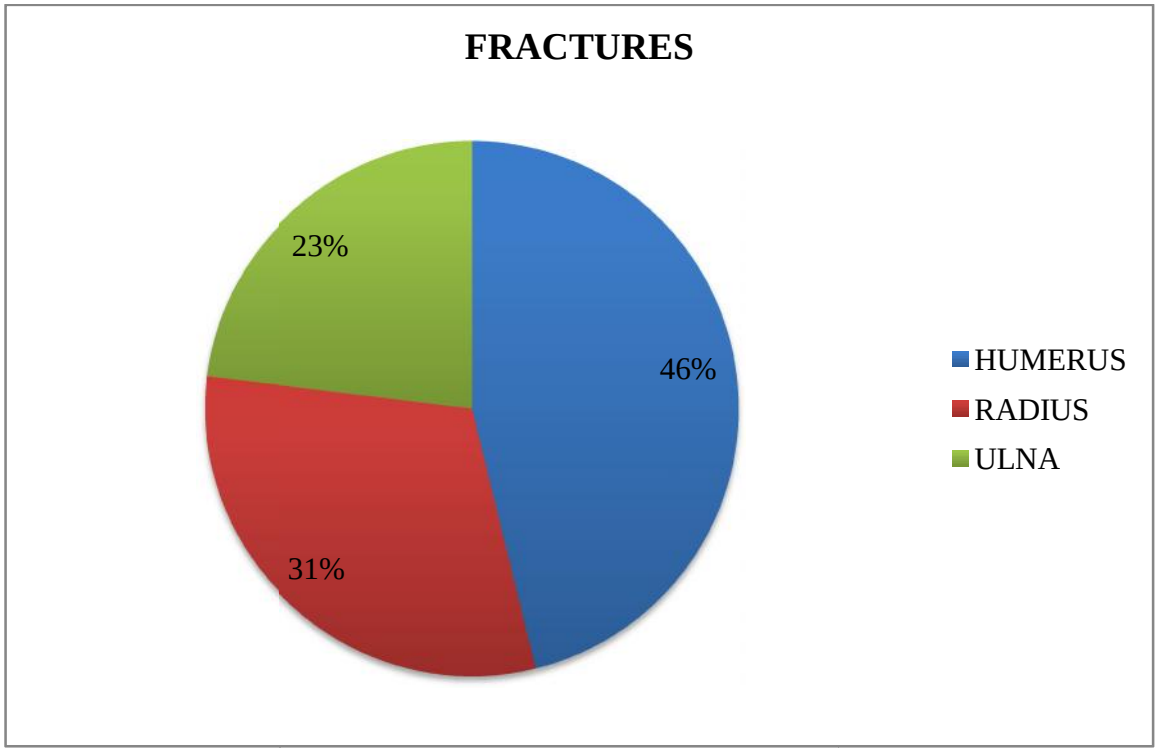


Figure XX: Pie diagram showing the distribution of fractures among the study subjects (n = 26)

Figure II represents a pie diagram showing the distribution of fractures among the study subjects. Most of the subjects had fracture of humerus (46.2%), followed by radius and ulna fractures.

TABLE 2: COMPARISON OF THE TYPES OF FRACTURES AMONG DIFFERENT AGE GROUPS (n=26)				
AGE GROUPS	**TYPES OF FRACTURES**			
	HUMERUS (%)	**RADIUS (%)**	**ULNA (%)**	**TOTAL (%)**
20 – 34 years	5 (45.4)	3 (27.3)	3 (27.3)	11 (100)
35 – 49 years	4 (44.4)	3 (33.3)	2 (22.2)	9 (100)
>50 years	3 (50)	2 (33.3)	1 (16.7)	6 (100)

Table 2 represents the comparison between the types of fractures among different age groups. The types of fractures were more among the 20 to 34 years age group and it showed an increasing trend with age advancement.

TABLE 3: COMPARISON OF THE TYPES OF FRACTURES AMONG THE STUDY AND CONTROL GROUPS (n=26)				
GROUPS	**TYPES OF FRACTURES**			
	HUMERUS (%)	**RADIUS (%)**	**ULNA (%)**	**TOTAL (%)**
STUDY GROUP	7 (53.8)	4 (30.8)	2 (15.4)	13 (100)
CONTROL GROUP	5 (38.5)	4 (30.8)	4 (30.8)	13 (100)

Table 3 represents the comparison between the types of fractures among the study and control groups. Fracture of humerus was reported more among both the study group (53.8%) and the control group (38.5%).

TABLE 4: DETECTION OF EARLY CALLUS FORMATION IN RELATION TO TIME IN WEEKS AMONG THE STUDY AND CONTROL GROUPS (n=26)			
DURATION	**STUDY GROUP (%)**	**CONTROL GROUP (%)**	**TOTAL (%)**
4TH WEEK	2 (66.7)	1 (33.3)	3 (100)
6TH WEEK	8 (100)	0 (0)	8 (100)
8TH WEEK	2 (25)	6 (75)	8 (100)
12TH WEEK	1 (16.7)	5 (83.3)	6 (100)
16TH WEEK	0 (0)	1 (100)	1 (100)
TOTAL	13 (50)	13 (50)	26 (100)

Table 4 shows the detection of early callus formation in relation to time in weeks among the study subjects. Most of the subjects in the study group had early callus formation in less than 8 weeks, whereas the callus had appeared in the control group after 8 weeks only.

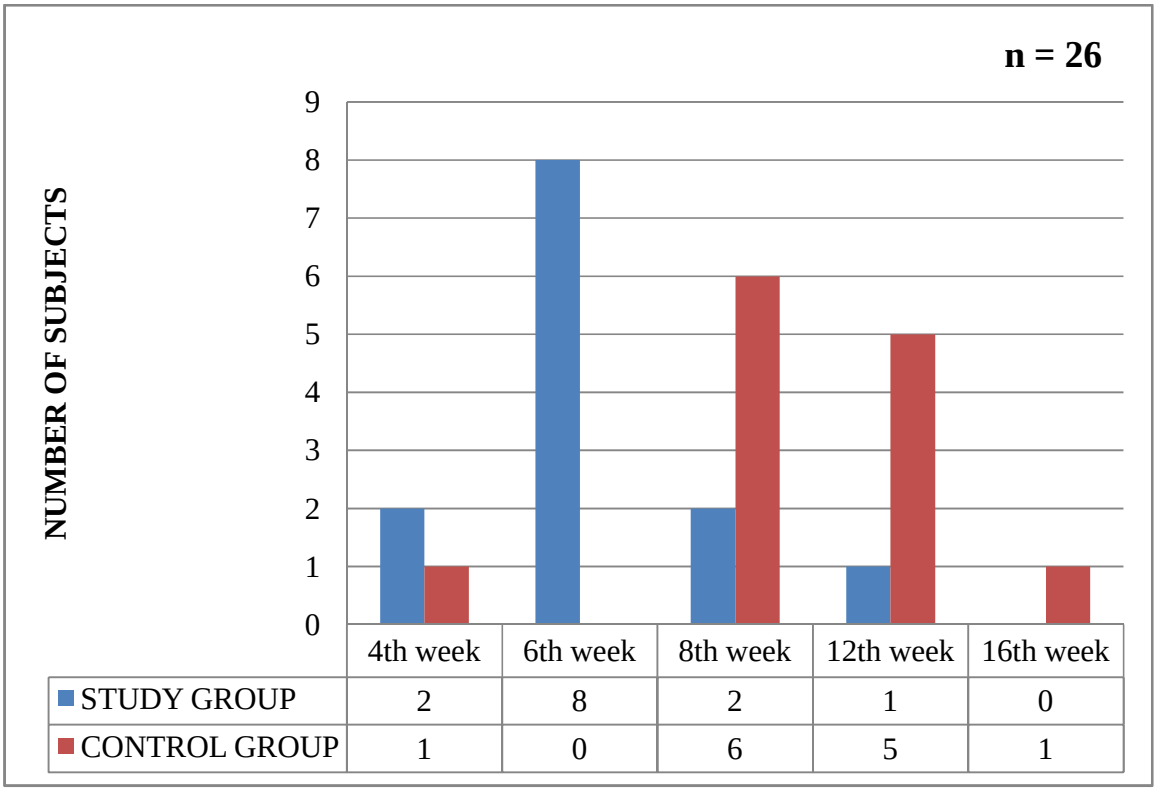


Figure XXI: Bar diagram showing the comparison of early callus formation among the study and control groups (n = 26)

Figure XXI explains that, majority of the study group had appearance of early callus in less than 6 weeks when compared to the control group.

TABLE 5: COMPARISON OF EARLY CALLUS FORMATION AMONG THE STUDY GROUP AND CONTROL GROUP (n=26)						
GROUPS	**4TH WEEK (%)**	**6TH WEEK (%)**	**8TH WEEK (%)**	**12TH WEEK (%)**	**16TH WEEK (%)**	**TOTAL (%)**
STUDY GROUP	2 (15.4)	8 (61.5)	2 (15.4)	1 (7.7)	0 (0)	13 (100)
CONTROL GROUP	1 (7.7)	0 (0)	6 (46.2)	5 (38.5)	1 (7.7)	13 (100)
p = 0.007*						

*Fisher's exact test

Table 5 represents the comparison among the study and control groups based upon the appearance of early callus. This table shows that the study group was statistically significant ($p<0.05$) when compared with the control group. 76.9% of the patients who received PRF+BCP showed early callus formation within the sixth week, whereas only 38.5% of the controls showed callus formation during their twelfth week.

DISCUSSION

A break in the continuity of a bone is known as a fracture. Injury to the musculoskeletal system results in damage to the bones, joints, tendons and muscles. There are many types of fractures and are classified based upon its pattern, aetiology and displacement. Fracture healing occurs in various stages, where callus formation is an essential part in the fracture healing process.

There are various platelet derived products or platelet concentrates which acts as biological mediators and facilitating the healing process. The latest developed platelet concentrates was Choukroun's PRF. The PRF is a second-generation platelet concentrate and is used to improve soft and hard tissue healing. It is an autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines. Therefore PRF has also been labelled as a healing biomaterial.

Biphasic calcium phosphate (BCP) is an alloplastic grafting product. It is synthetic in origin, osteoconductive, osteoinductive and biocompatible [21]. The calcium ions are released in a controlled manner which is favourable for the formation of an apatite layer. The apatite layer helps in bioactivity of the BCP. Therefore clinically applied PRF with BCP was used in this study.

PRF is widely used in dental surgeries. But, it is not commonly used in Orthopaedics. In our study we have used PRF in combination with BCP to evaluate upper limb diaphyseal fractures.

The present study was to evaluate the efficacy of fracture healing by using PRF in combination with BCP among the study group and was compared with a control group. After obtaining human ethical clearance from our institution and informed consent from the patients the study was performed. The study was performed for duration of 2 years.

Tatullo et al, conducted histological and clinical evaluations of 60 patients who underwent sinus lifting surgery before implant surgery. The experimental group received bovine bone graft material (Bio-Oss) combined with PRF, whereas the control group received only bovine bone graft material (Bio-Oss, Geistlich Pharma AG, Wolhusen, Switzerland). In contrast, our study was conducted by using the combination of PRF with BCP alone among 13 subjects who were compared with a group of 13 controls.

There are various studies conducted on animal models only. Our study was conducted on humans after taking their informed consent. Ozdemir et al assessed the effects of PRF on bone augmentation in an animal model. Surgically created defects were filled with PRF, BCP, or anorganic bovine bone (ABB) and were covered with titanium membranes. Control groups were left empty. Histomorphometric evaluation was carried out by Ozdemir et al at 1 and 3 months and Tatullo et al evaluated it on 106, 120 and 150 days. On contrary, radiological evaluation was carried out every month till the fracture healed in our study.

In the study by Ozdemir et al, the control group had the least new bone formation, and new bone formation was seen among PRF, BCP, and ABB groups after 1 month. PRF and ABB groups had a better new bone formation at 3 months. Zhang et al revealed that there was no difference in the new bone between the group receiving only bovine bone graft (Bio- Oss) and that receiving PRF in combination with bovine bone graft 6 months after sinus-lifting surgery.

Contrary to the result of this study, we observed statistically higher significance in the group with fractures filled with PRF + BCP than the control group on 4^{th} and 6^{th} week. Similar to our study, the studies conducted by Abdelmagid et al, and Bolukbasi et al, showed an increase in bone formation with addition of PRF to BCP.

These results prove that PRF in combination with BCP was effective in the early stages of fracture healing.

RECOMMENDATIONS

1. PRF is a cost effective platelet concentrate
2. PRF acts as a biological adhesive which adheres the bone graft material together
3. The gelatinous consistency of PRF favors the clot stability and its membranous shape provides a natural barrier effect
4. BCP which has both osteoconductive and osteoinductive properties acts as a bone scaffold when combined with PRF
5. PRF provides vascularization when combined with a bone graft which helps in the survival of the graft.

LIMITATIONS

1. The sample size of the study was limited as there was inadequacy of cases.
2. Inter and intra observer reliability while interpreting the radiographs.
3. Patients were not analysed until complete fracture healing.

CONCLUSION

The total subjects participated in our study were 26. Among them 13 patients received PRF + BCP in addition to the current standard of care. They were compared with a control group of 13 patients who received only the current standard of care.

The overall mean age & standard deviation was 38.69±13.97 years. Most of the subjects (42.3%) were in the age group of 20 to 34 years and majority of the subjects had fracture of humerus (46.2%), followed by radius and ulna fractures. Fracture of humerus (53.8%) was reported more among both the cases and controls.

Radiological evaluation was carried out every month till the fracture had healed. There was a statistically higher significance in the group with fractures filled with PRF + BCP than the control group on 4^{th} and 6^{th} week. 92.3% of the cases showed callus formation in less than 8 weeks, whereas 46.2% of the controls showed callus formation during their 12^{th} week only.

PRF is easy to prepare. Being an autologous preparation, it has the least adverse reactions.PRF has its best effect when left undisturbed for a week since it has the property of slow release of growth factors.

From the results of this study, we can conclude that, PRF in addition to BCP favours the early callus formation and improves fracture healing process. The effectiveness of PRF depends not only on its features but also the properties of co-administered grafting material.

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ANNEXURES

PSG Institute of Medical Science and Research, Coimbatore

Institutional Human Ethics Committee

INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

We (write name of the investigator(s) here), Dr. B.K.Dinakar Rai, Dr. R.Balachandran are carrying out a study on the topic:

as part of my / our research project being carried out under the aegis of the Department of:

(*Applicable to students only*): My / our research guide is: Dr. B.K.Dinakar Rai

The justification for this study is:

- Various bone substitutes have been introduced for bone augmentation, among the variety of grafting materials, PRF has become a focus of current studies due to its potential to accelerate and improve the healing process.
- The advantages of BCP compared with autogenous grafts are their synthetic origin, biocompatibility, osteoconductivity and avoidance of a second surgical site. Therefore BCP is preferred in this study.

- In the present study, we are going to evaluate PRF in combination with BCP in fracture healing.

The objectives of this study are:

Primary Objective: To evaluate the efficacy of Platelet Rich Fibrin mixed with Biphasic calcium phosphate in fracture healing.

Secondary Objective:

Sample size: __26 patients__.

Study volunteers / participants are (specify population group & age group): 18 years and above. Participants would be patients who come under the inclusion criteria of the study.

Location: PSG Hospitals, Coimbatore

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration):_____ minutes.

Data collected will be stored for a period of 2 years. We will / will not use the data as part of another study.

Health education sessions: Number of sessions: _____. Approximate **duration** of each session:

_____ minutes.

Clinical examination (Specify details and purpose):

Blood sample collection: Specify quantity of blood being drawn: 80 ml.

No. of times it will be collected: Once.

Whether blood sample collection is part of routine procedure or for research (study) purpose:

1. Routine procedure ✓2. Research purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: Nil

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

Medication given, if any, duration, side effects, purpose, benefits:

Whether medication given is part of routine procedure: Yes / No (If not, state reasons for giving this medication)

Whether alternatives are available for medication given: Yes / No (If not, state reasons for giving this particular medication)

Final interview (specify approximate duration):_____ mts. If **photograph** is taken, purpose:

Benefits from this study: Improved Fracture healing

Risks involved by participating in this study: Nil

How the **results** will be used: Results will be sent to T.N. Dr. MGR University of Health Sciences for dissertation purpose and for publication.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict

confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings- including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date:

Witness:

Contact number of PI: 9894140052

Contact number of Ethics Committee Office: During Office hours: 0422 2570170 Extn.: 5818

After Office hours: 9865561463

Annexure - IV
ஒப்புதல் படிவம்

ஆர்.பாலசந்திரன் ஆகிய நான், PSG மருத்துவக் கல்லூரியின் எலும்பு முறிவுத் துறையின் கீழ் **"EFFICACY OF PLATELET RICH FIBRIN IN COMBINATION WITH BIPHASIC CALCIUM PHOSPHATE IN FRACTURE HEALING"** என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி: டாக்டர்.பி.கே.தினகர் ராய் D.Ortho/ M.S.Ortho
ஆய்வு மேற்கொள்வதற்கான அடிப்படை:

ஆய்வின் நோக்கம் :

ஆய்வில் பங்கு பெரும் நபர்களின் எண்ணிக்கை:

ஆய்வு மேற்கொள்ளும் இடம் :

ஆய்வின் பலன்கள் :

ஆய்வினால் ஏற்படும் அசௌரியங்கள் / பக்க விளைவுகள் :

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் ---------- வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்தப்பட மாட்டாது. எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது. அவை இரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக்கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்குக் கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக்கொள்ளும்

உரிமை தங்களுக்கு உண்டு. ஆய்விலிருந்து விலகிக்கொள்ளும் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்தவித மாற்றமும் இருக்காது.

இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கப்படும் , சில இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுக்கப்படும்.

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப்படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :
தேதி:

ஆய்வுக்குட்படுபவரின் ஒப்புதல் :
நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினைப் பற்றி தெளிவாகவும், விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும், இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி:

கையொப்பம் :
தேதி:

ஆய்வாளரின் தொலைபேசி எண்
மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண் : 0422 2570170 Extn: 5818

MASTER CHART

NAME	OP_NO	IP_NO	GROUPS	AGE	GENDER	HUMERUS	RADIUS	ULNA	FRACTURES	AGE_ GROUP	CALLUS_ WEEKS	WEEKS_ CAT
AMALAN	O17035131	I17018510	1	34	1	1	0	0	1	1	6	1
VASANTHAKUMARI	O16069276	I16030977	1	45	2	2	0	0	1	2	6	1
KALAIVANI	O10029220	I16039202	1	26	2	0	1	0	2	1	6	1
NAVAMANI	O17008785	I17005141	1	25	2	2	0	0	1	1	4	1
TAMILVANAN	O16036471	I17005578	1	24	1	2	0	0	1	1	6	1
SOWMITHA	O17026042	I17013462	1	42	2	2	0	0	1	2	4	1
SOCKALINGAM	O17026311	I17013578	1	53	1	1	0	0	1	3	8	1
KUMARASAMY	O16059213	I16026774	1	45	1	0	2	0	2	2	12	2
SHANMUGASUNDARAM	O16068843	I16030756	1	41	1	2	0	0	1	2	6	1
PADMAVATHI	O16085379	I16038832	1	55	2	0	0	2	3	3	6	1
SANGEETHA_1	O17021057	I17011453	1	29	2	0	0	2	3	1	6	1
SANGEETHA_2	O17021057	I17011453	1	29	2	0	2	0	2	1	8	1
SUBBAMMAL	O17000413	I17000321	1	70	2	1	0	0	1	3	12	2
RAMANAN	O17047021	I17026080	2	43	1	0	0	1	3	2	8	1
SATHISH	O16017080	I16008124	2	21	1	1	0	0	1	1	16	2
KANNAN	O16038702	I16018305	2	40	1	0	0	2	3	2	12	2
RAMBABU	O16042892	I16020191	2	35	1	2	0	0	1	2	12	2
HARIHARAN	O16021881	I16010413	2	21	1	1	0	0	1	1	8	1
TAMILMANI	O16039149	I16018551	2	60	1	2	0	0	1	3	12	2
SASITHARAN_1	O16040713	I16018430	2	22	1	0	1	0	2	1	12	2
VIJAYA	O17039573	I17021260	2	53	2	0	2	0	2	3	8	1
RAMANAN_1	O17047021	I17026080	2	43	1	0	1	0	2	2	4	1
LAKSHMI	O07062315	I16013581	2	59	2	2	0	0	1	3	8	1
VIJAYAKUMAR	O16027118	I16013032	2	45	1	0	2	0	2	2	8	1
SIVA	O16032132	I16015411	2	24	1	0	0	1	3	1	12	2
SASITHARAN_2	O16040713	I16018430	2	22	1	0	0	1	3	1	8	1