

**FABRICATION AND CHARACTERIZATION OF A NOVEL BONE GRAFT
MATERIAL CONTAINING BIPHASIC CALCIUM PHOSPHATE FORTIFIED WITH
THE SEED ETHANOLIC EXTRACT AND SEED POWDER OF *OCIMUM
BASILICUM***

Submitted in partial fulfilment of the requirements for the award of a Bachelor of
Technology in Biomedical Engineering.

By

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**DEPARTMENT OF BIOMEDICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **GEETHANJALI.G (39240017)**, **SHEEBA SRI.B (39240036)** who carried out the final year project entitled "FABRICATION AND CHARACTERIZATION OF A NOVEL BONE GRAFT MATERIAL CONTAINING BIPHASIC CALCIUM PHOSPHATE FORTIFIED WITH THE SEED ETHANOLIC EXTRACT AND SEED POWDER OF *OCIMUM BASILICUM*" under our supervision from October to April 2023

Dr. J. Bethanne Janney

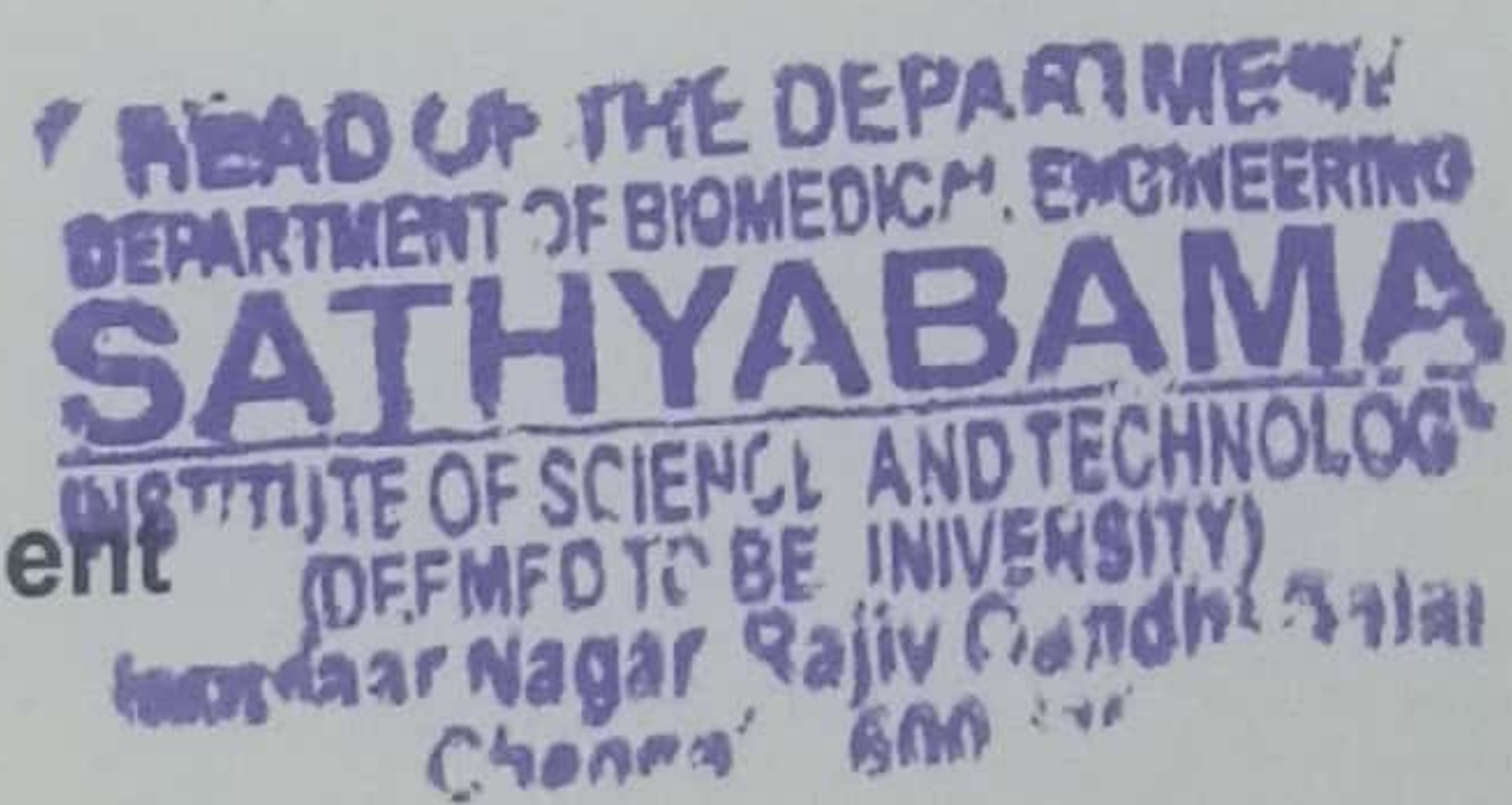
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DECLARATION

We, **GEETHANJALI G (39240017) AND SHEEBA SRI B (39240036)** AND hereby declare that the project report entitled "**FABRICATION AND CHARACTERIZATION OF A NOVEL BONE GRAFT MATERIAL CONTAINING BIPHASIC CALCIUM PHOSPHATE FORTIFIED WITH THE SEED ETHANOLIC EXTRACT AND SEED POWDER OF *OCIMUM BASILICUM***" done by us under the guidance of **Dr. J. BETHANNEYJANNEY**, Associate Professor, Department of Biomedical Engineering and **DR. S. SRIVIDYA**, Associate Professor, Department of Bio chemistry, Sathyabama Dental College and Hospital at Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in B Tech Biomedical Engineering.

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ABSTRACT

Herbal bone grafts have gained momentum in proving its osteogenic properties among which *Ocimum basilicum* L (Ob), a versatile plant commonly called as Basil is a promising plant with anti-inflammatory, anti-microbial, anti-oxidant and anti-arthritis activity. The present study, incorporates the ethanolic seed extract of Ob into a bone graft containing biphasic calcium phosphate (BCP) and the Ob seed powder. The fabricated bone graft (BCP-Ob) was subjected to various physicochemical characterization namely FTIR, XRD, SEM, and TGA. The extract was subjected to GCMS and invitro cell culture studies to reveal its bioactive compounds and biocompatibility. The results revealed that the incorporation of Ob seed powder creates a porous matrix for the graft which was shown in the SEM. FTIR and XRD results revealed the presence of peaks and 2θ values for hydroxyapatite and β Tricalcium phosphate in the graft respectively. The thermogravimetric analysis revealed its stability and the inorganic content of the herbal graft. Hence, the novel herbal bone graft (BCP-Ob) proves to be a promising graft and thus it can be taken further for invitro and invivo evaluation for biomedical applications.

Keywords - *Herbal Bone graft, Hydroxyapatite, Tricalcium phosphate, Ocimum basilicum, Biphasic calcium phosphate, Basil seed.*

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	FULL FORM
1	Ob	<i>Ocimum basilicum</i>
2	HAP	Hydroxyapatite
3	β TCP	Beta Tricalcium Phosphate
4	BCP	Biphasic Calcium Phosphate
5	FTIR	Fourier Transform Infrared Spectroscopy
6	GCMS	Gas Chromatography and Mass Spectrometry
7	SEM	Scanning Electron Microscopy
8	TGA	Thermo Gravimetric Analysis

CHAPTER 1

INTRODUCTION

Bone is a mineralized connective tissue that exhibits four types of cells: osteoblasts, bone lining cells, osteocytes, and osteoclasts. Bone exerts important functions in the body, such as locomotion, support and protection of soft tissues, calcium and phosphate storage, and harboring of bone marrow. Despite its inert appearance, bone is a highly dynamic organ that is continuously resorbed by osteoclasts and new formed by osteoblasts. There is evidence that osteocytes act as mechanosensors and orchestrators of this bone remodelling process. The function of bone lining cells is not well clear, but these cells seem to play an important role in coupling bone resorption to bone formation.

Bone remodelling is a highly complex process by which old bone is replaced by new bone, in a cycle comprised of three phases; initiation of bone resorption by osteoclasts, the transition (or reversal period) from resorption to new bone formation, and the bone formation by osteoblasts. This process occurs due to coordinated actions of osteoclasts, osteoblasts, osteocytes, and bone lining cells which together form the temporary anatomical structure called basic multicellular unit (BMU).

Normal bone remodelling is necessary for fracture healing and skeleton adaptation to mechanical use, as well as for calcium homeostasis. On the other hand, an imbalance of bone resorption and formation results in several bone diseases. For example, excessive resorption by osteoclasts without the corresponding amount of new formed bone by osteoblasts contributes to bone loss and osteoporosis, whereas the contrary may result in osteopetrosis. Thus, the equilibrium between bone formation and resorption is necessary and depends on the action of several local and systemic factors including hormones, cytokines, chemokines, and biomechanical stimulation.



Fig 1.1: Human Skeleton

The function of bone tissue is both supportive and locomotive (Roia P et.al., 2021). Bones not only provide the physical scaffold of the body but enable locomotion by providing attachment sites for muscles, tendons and ligaments (Selhotra A et.al., 2020). In addition, bones permit motor capability, protect vital organs, facilitate breathing, play a role in homeostasis, and produce a variety of cells in the marrow critical for survival (Cowan PT et.al., 2022). Bone tissue is unique in that it can regenerate, restoring to a fully-functional, pre-injury state.

Bone is a metabolically active connective tissue that provides structural support, facilitates movement, and protects vital organs. Bone is composed of an extracellular matrix and bone cells (osteocytes) (El sayed SA et.al., 2019). Under the right conditions, bone tissue undergoes a process of mineralisation, and is hardened by deposited calcium. Bone is hard and many of its functions depend on that characteristic hardness.

The adult human skeleton is composed of 206 bones. At birth, there are approximately 270 bones, with the final adult count decreasing as a portion of these bones fuse during phases of skeletal growth and maturation. During life, bone is constantly remodelling with most of the adult skeleton being replaced about every 10 years.

All bones are mainly divided into two categories: cancellous bones and cortical bones. Out of these two types of bones, cortical bones make up most of the skeletal system (up to 80%), while the rest is cancellous bones (Singh S et.al., 2018).

It also includes anatomy of the skeletal system, including the flat, short, and irregular bones, and their individual arrangements of compact and spongy

1.1 BONE STRUCTURE

1.1.1 *Bone consists of two types of tissue*

1. Compact (cortical) bone: makes up 80% of the total bone in the body and is much stronger than cancellous/trabecular bone. It is very resistant to bending, torsion, and compression. It is much more dense and has a minimal role in metabolism. It is seen mostly in the shaft of long bones like the femur and the tibia as well as in the outer shell of trabecular bone
2. Cancellous (trabecular or spongy) bone: makes up only 20% of the total bone but has ten times the surface/volume ratio of cortical bone. It responds eight times faster to changes in load making it far more dynamic. It occurs in areas that are more subject to compression such as the vertebral body, pelvis, and the metaphyses (Daig NA et.al., 2019)

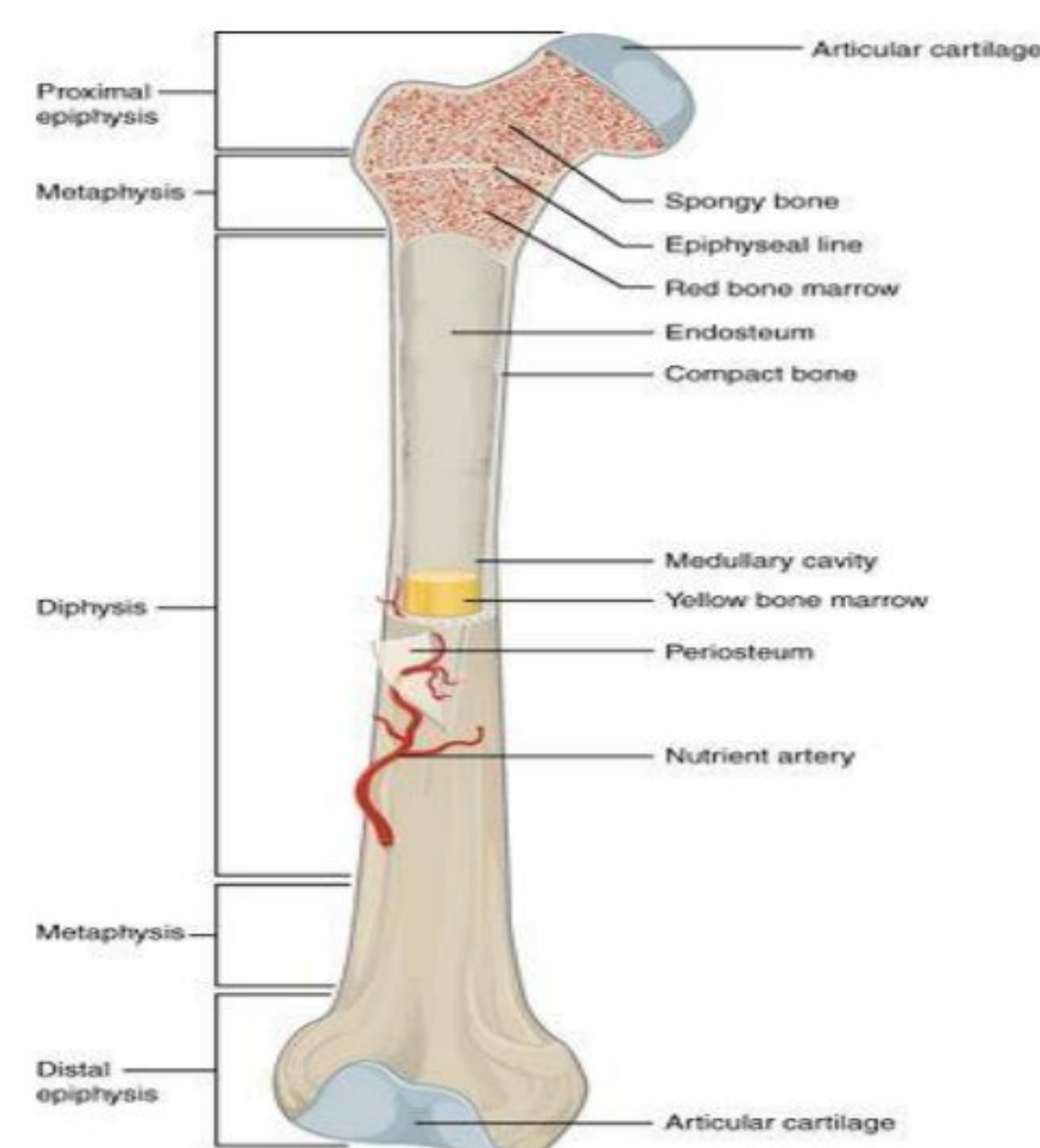


Fig 1.2: Bone Structure

1.1.2 Bones also contain

- Osteoblasts and osteocytes, responsible for creating bone
- Osteoclasts or bone resorbing cells
- Osteoid, a mix of collagen and other proteins
- Inorganic mineral salts within the extracellular matrix (ECM)
- Nerves and blood vessels
- Bone marrow
- Cartilage
- Membranes, including the endosteum and periosteum

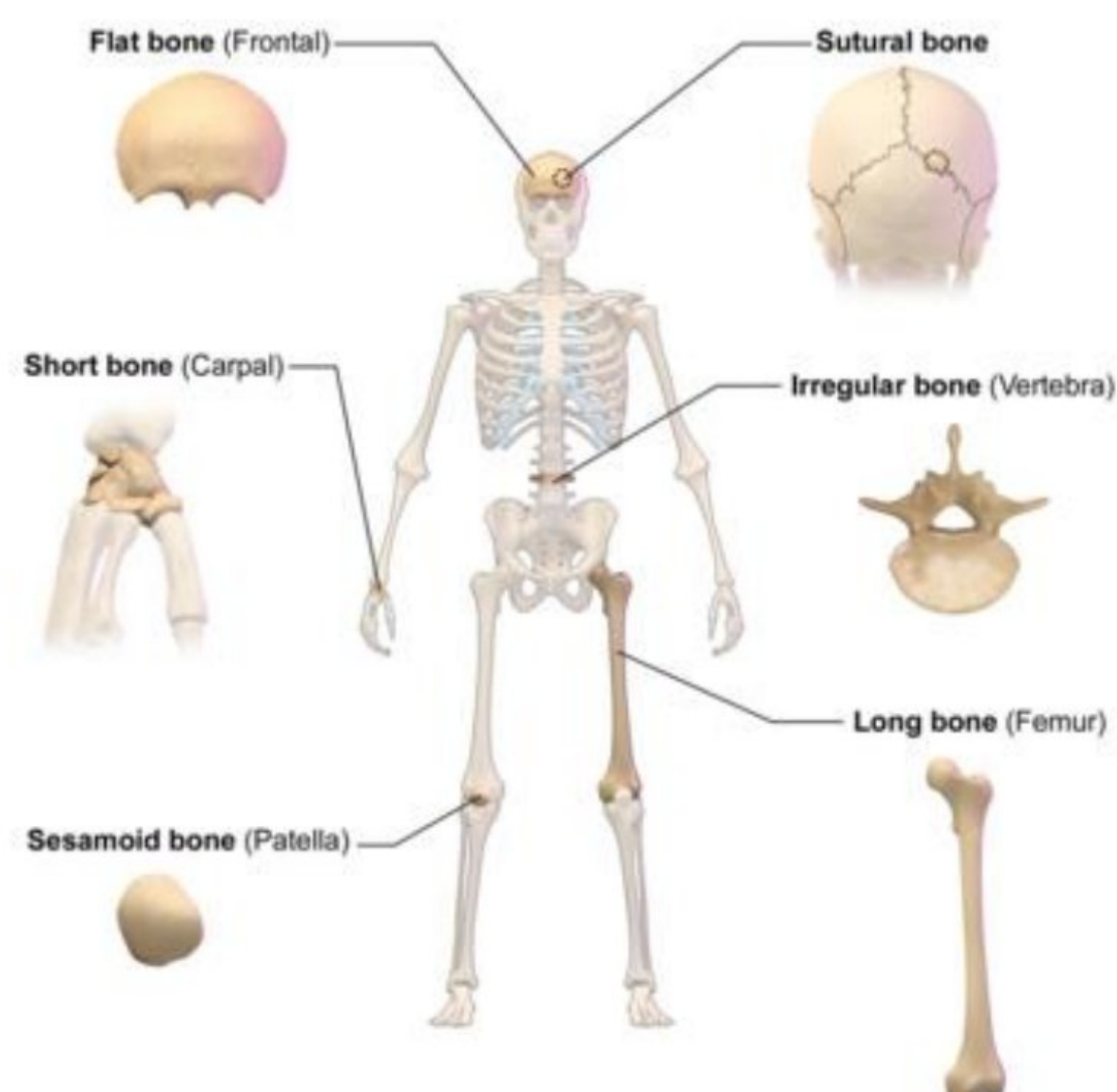


Fig 1.3: Classification of Bones

In the human body, five bone types exist:

1. Long bones: Mostly made of compact bone, and have little marrow. Most bones in the limbs are long bones. These bones

tend to support weight and help movement (e.g., femur, tibia, fibula, humerus).

2. Short bones: Have only a thin layer of compact bone (e.g. bones of the wrist and ankle).
3. Flat bones: Usually bones that are thin and curved. They consist of two outer layers of compact bone and an inner layer of spongy bone. Flat bones include most of the bones of the skull and the sternum or breastbone. They tend to have a protective role (e.g. sternum, ribs).
4. Sesamoid bones: These are embedded in tendons (e.g, patella). They protect tendons from wear and stress.
5. Irregular bones: As their name implies, these are bones that do not fit into the first four categories and have an unusual shape. They include the bones of the spine and pelvis. They are often protecting organs or tissues.

1.2. GROSS ANATOMY

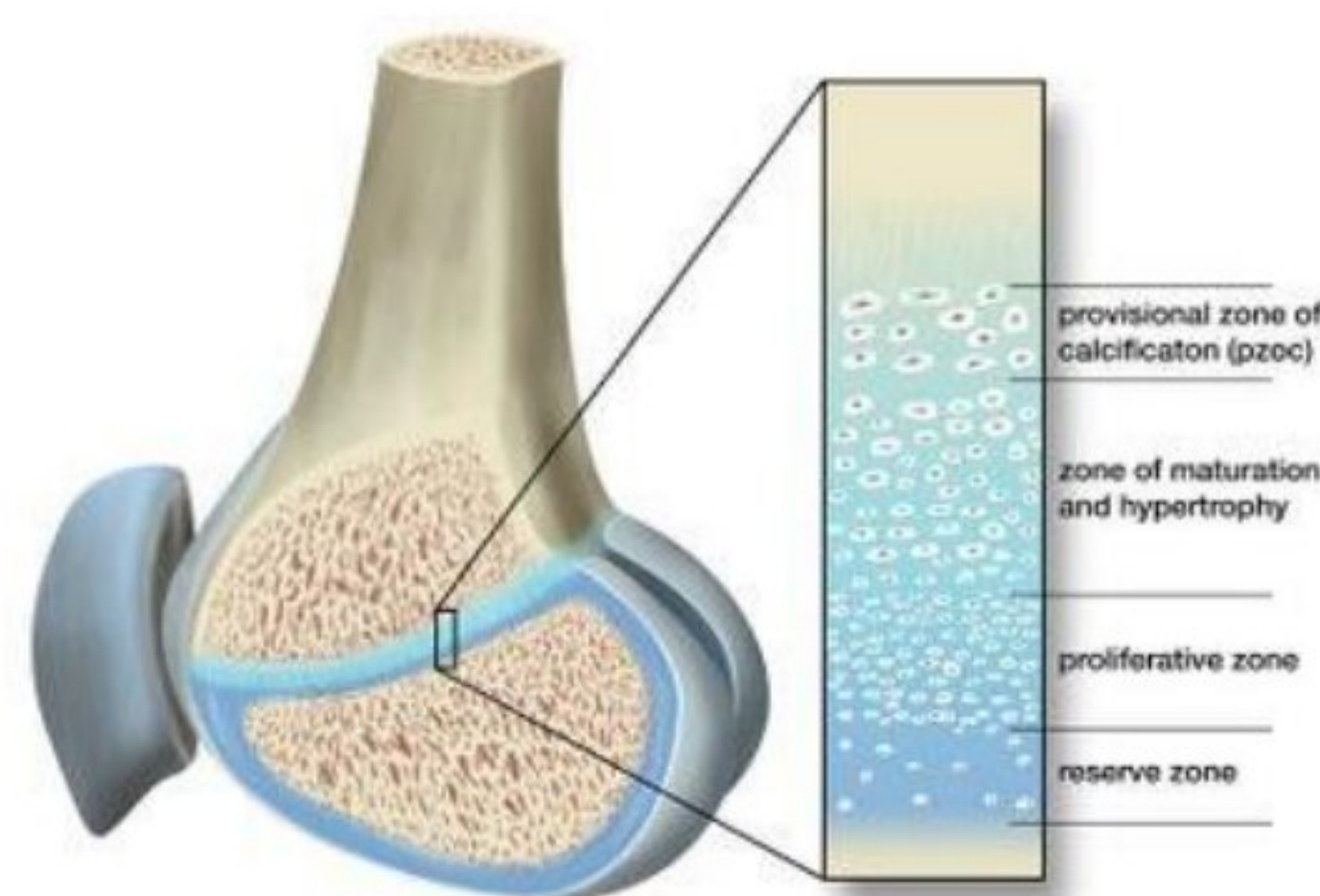


Fig 1.4: Physeal anatomy

A long bone has two parts, the diaphysis and the epiphysis. Bones that undergo endochondral ossification also have a physis (growth plate).

1. The diaphysis is the tubular shaft that runs between the proximal and distal ends of the bone. The hollow region in the diaphysis is called the medullary cavity, which is filled with yellow marrow. The walls of the diaphysis are composed of dense and hard compact bone (Hall JE et.al., 2015)

2. The physes (singular: physis) or growth plates are found in bones that undergo endochondral ossification (the process by which the embryonic cartilaginous model of most bones contributes to longitudinal growth and is gradually replaced by bone)
3. The epiphysis - the part or process of a bone that ossifies separately and later becomes ankylosed to the main part of the bone. The epiphysis contributes to a joint, compared with an apophysis which is a site of tendon or ligament attachment. Once the growth plate has fused, the epiphysis and metaphysis are joined.

1.3 BONE COMPOSITION

Bone is composed of cells and the extracellular matrix (ECM), which has both organic and inorganic substances. Osteoblasts synthesis bone.

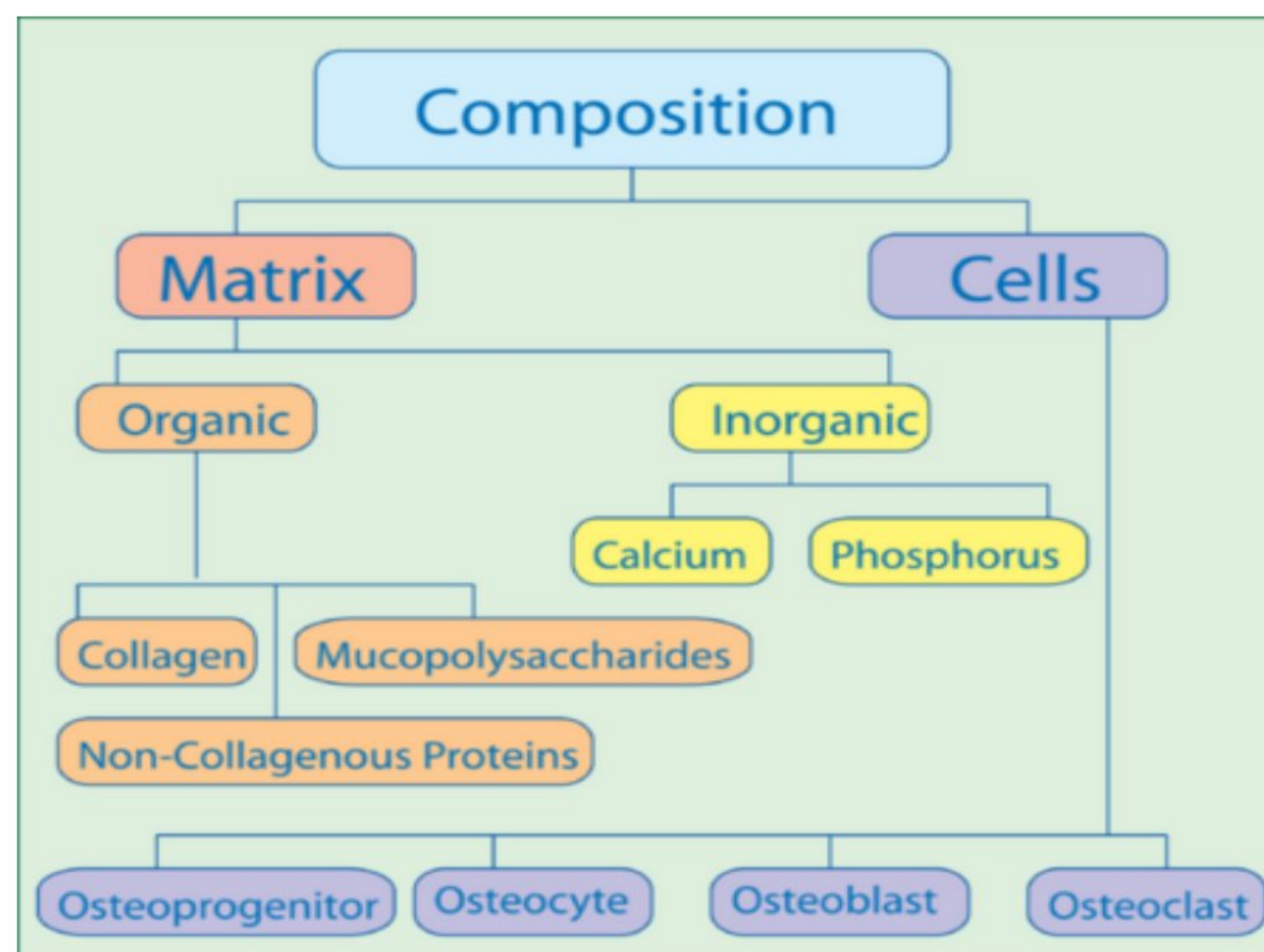


Fig 1.5: Composition of Bone

1.4 ECM

The ECM consists of:

1. Type I collagen mixed with a matrix of calcium phosphate crystal (which is up to 70 % of the dry weight)
2. Proteoglycans and glycoproteins, which are less abundant, but vital for the organization of collagen fiber mineralisation and resorption of bone. Chondroitin sulfate accounts for 67-97 % of the bone glycosaminoglycans.

Bone cells make up about 10% of total bone volume. Bones are not a static tissue but need to be constantly maintained and remodelled. There are three main cell types involved in this process.

1.5 BONE CELLS

Osteoblasts: Bone marrow stem cells can differentiate into osteoblasts. Osteoblasts are responsible for making new bone and repairing older bone. Osteoblasts produce a protein mixture called osteoid, which is mineralised and becomes bone. Another function is to manufacture hormones, including prostaglandins.

1. **Osteocytes:** Inactive osteoblasts that have become trapped in the bone that they have created. They maintain connections to other osteocytes and osteoblasts. They are important for communication within bone tissue.
2. **Osteoclasts:** Large cells with more than one nucleus, which break down bone. They release enzymes and acids to dissolve minerals in bone and digest them in a process called resorption. Osteoclasts help remodel injured bones and create pathways for nerves and blood vessels to travel through.

1.6 EXTRACELLULAR MATRIX

Bones are, in reality, living cells embedded in a mineral-based organic matrix. This extracellular matrix consists of:

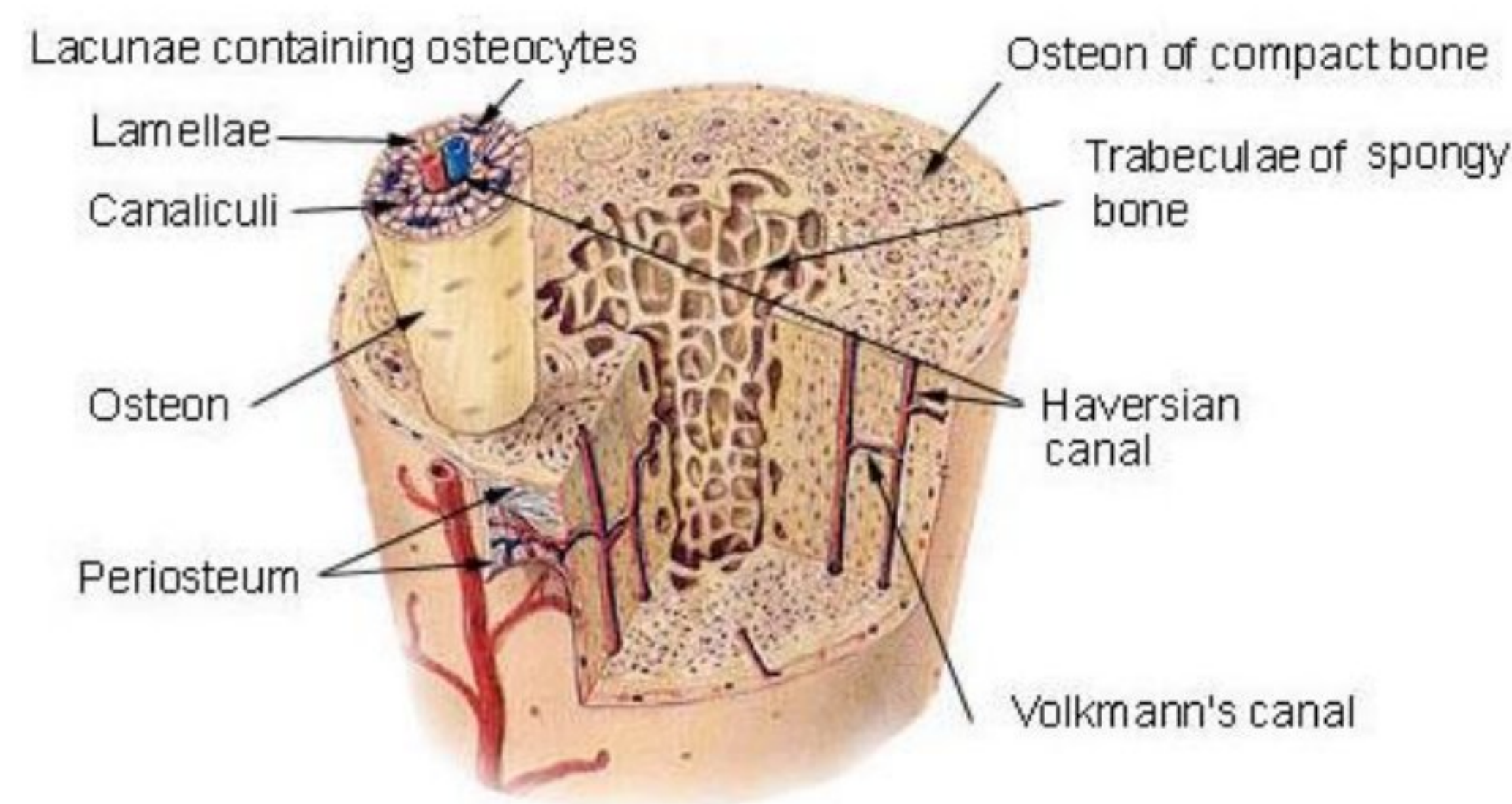
- organic components, being mostly type 1 collagen

- inorganic components, including hydroxyapatite and other salts, such as calcium and phosphate

Collagen gives bone its tensile strength, namely the resistance to being pulled apart. Hydroxyapatite gives bones compressive strength or resistance to being compressed

1.7 BONE MARROW AND HEMATOPOIESIS

Bone marrow is found in almost all bones where cancellous bone is present. Bone marrow is responsible for hematopoiesis, or the production of red blood cells, white blood cells, and platelets. It makes around 2 million red blood cells every second, as well as producing platelets, and white blood cells. Defective



and old red blood cells are also destroyed in the bone marrow.

Fig 1.6: Compact and Spongy bone

1.8 FUNCTIONAL UNIT OF COMPACT BONE

Osteons represents a single functional unit of bone tissue. It is arranged with concentric lamellae of collagen fiber orientations around a central canal "consisting of osteocyte's arterial, venous, and nerve supply", which is known as the Haversian canal. This system also includes the canaliculi and Volkmann canal. These allow for communication between neighboring osteocytes and osteons, respectively. Osteons are several millimeters long and about 0.2

millimeter (0.008 inch) in diameter; they tend to run parallel to the long axis of a bone.

1.9 MECHANICAL FUNCTIONS

Bones are vital for protecting the important and fragile organs in the body. For example bones protect the heart and the brain.

1. Structure. Without bones, our body would have no frame and essentially be an immobile lump of flesh and tissue.
2. Movement. The bones pair up with the joints, ligaments, tendons and muscles to allow the body to move as it does.
3. Sound Transduction. Bones are also important for conduction vibrations which allow us to hear.

1.10 METABOLIC FUNCTIONS

The metabolic functions of the bone are many:

The bone matrix can store several minerals, chiefly calcium and phosphorus as well as iron in the form of ferritin.

1. Chondroitin sulfate, a carbohydrate moiety, is also a commonly found element in the matrices.
2. Specific growth factors, including insulin-like growth factor or IGF-1, are housed in bone and then released periodically.
3. pH balance is also regulated as bones may alter the composition of alkaline salts in the serum to maintain the optimal pH level.
4. Osteocytes can engulf toxic molecules and heavy metals from the serum as a means of detoxification.
5. Fat storage.

1.11 REMODELLING

This is a physiological process in which old or damaged bone is removed by osteoclasts and then replaced by new bone formed by osteoblasts (Epsky S et.al., 2021) (Ofer N et.al., 2019). There is a tight coupling of bone formation to bone resorption to ensure no net change in bone mass or quality after each remodelling. It requires coordinated action of the four types of bone cells. The process involves four major distinct but overlapping phases:

1. Initiation/activation of bone remodelling at a specific site. The osteoclast precursors are recruited to bone remodelling compartments (BRC).
2. Bone resorption and concurrent recruitment of osteoprogenitors. Bone resorption represents the predominant event, but the recruitment of mesenchymal stem cells (MSCs) and/or osteoprogenitors into the BRC is also initiated.
3. Osteoblast differentiation and function (osteoid synthesis). Excavated bone is replaced with osteoid produced by osteoblasts.
4. Mineralisation of osteoid and completion of bone remodelling. The osteoid is mineralised, and the bone remodeling cycle is conclude

1.12 CLINICAL SIGNIFICANCE

Bone tissue is susceptible to a myriad of pathologies that may range from aetiologies of embryological, metabolic, autoimmune, neoplastic, or idiopathic origins. These include, but are not limited to, the conditions discussed below.

- Achondroplasia is a genetic disorder commonly associated as a cause of dwarfism. Individuals affected may present with short extremities due to decreased development of endochondral bone.
- Paget disease of the bone is characterized to be an imbalance amongst the activities of osteoblasts and osteoclasts. Of unknown aetiology, the condition only affects localized portions of the skeletal

tissue, generally involving one or more neighboring bones rather than the whole skeletal system.

- If untreated, Paget disease of the bone can act as a risk factor for osteosarcoma, a malignant proliferation of osteoblasts.
- Skeletal neoplasm begins in the metaphysis of long bones. Patients may report bone pain with swelling or a pathologic fracture may occur (i.e. a break in the bone caused by weakness of the bone through disease rather than trauma). This is much more common in teenagers than in the elderly.
- Bone fractures
- Osteoporosis. This is a common disorder of bone remodelling which is characterized by low bone mass and structural deterioration of bone. It causes bone fragility and increased vulnerability to fractures.
- Osteoarthritis
- Osteomalacia
- Rickets
- Epiphyseal plate disorders

1.13 FRACTURES

When significant force or too much pressure is applied to a normal bone results in a condition called fracture. Fractures are usually caused by a fall, bending or twisting of the bone or strike from an object. In some clinical conditions like osteoporosis, where the bone is weakened, fracture occurs or when there is excessive bone remodelling such as Paget's disease fracture occurs. Fractures are classified in to incomplete and complete fractures. Incomplete fracture is the type where bone is partially broken or cracked. Hair line fracture is an example of incomplete fracture. This kind of fracture happens due to a minor injury. Complete fracture or simple fracture in which the bone is broken into two fragments.

Comminuted fracture also called multifragmentary fracture occurs due to a severe injury in which the bone is broken into several fragments. Segmental fracture results in the requirement of a new bone to replace the lost bone. Repairing these bone defects has become the main goal in many clinical fields. To treat the bone

defects several methods in bone regeneration are available (Meyer and Wiesmann 2006) such as autologous bone, xenografts, calcium-phosphate bone graft substitute, demineralized bone matrix, autologous bone marrow, distraction osteogenesis etc.

Autologous bone grafts are treated as the gold standard for bone regeneration (Ewers et.al., 2004). The conventional method of harvesting the autologous bone graft from iliac crest is the standard procedure compared to all other bone graft substitutes. However, this method has some disadvantages in donor availability and donor site morbidity (Frohlich et. al., 2008).

Recent development in alternating the autologous bone grafts have arrived by using demineralized bone matrix, recombinant growth factors, combining biomaterials and growth factors (Calvert et. al., 2003). Bone regeneration is done using the three main elements namely osteogenesis, osteoconduction and osteoinduction. These elements are coupled with osteointegration which leads to perfect bone regeneration. Osteointegration is the interaction and bonding of the host bone with the bone graft (Giannoudis et. al., 2005).

Bone growth factors such as TGF – β (Glowack et. al., 2005), beta Fibroblast Growth Factor (Ornitz 2005) and BMPs (Yoon and Lyons 2004) regulates the replication, differentiation and function of bone cells (Canalis et. al., 2003). Bone morphogenetic proteins (BMPs) and stimulate the differentiation of osteoblastic cells into mature osteoblast. BMP-2 act as a bone stimulator during bone morphogenesis (Fromigue et. al., 2004).

1.14 FRACTURE HEALING

Fracture healing or bone healing is a three-step process (i.e.) reactive phase, reparative phase and remodelling phase. After fracture, blood vessels constrict and stops bleeding. The extravascular blood cells form a blood clot called hematoma. The clotting mechanism activates chemotaxis, thereby infiltration of inflammatory, endothelial and mesenchymal cells causes the formation of granulation tissue.

In the reparative phase, differentiation of mesenchymal cells occurs leading to the formation of a new mineralizing matrix. These cells are formed from periosteum near the fracture site and also from the distant soft tissues through blood stream. The combination of endochondral and intramembranous ossification leads to the formation of new bone tissue. The fracture site is stabilized by cartilaginous matrix. This newly formed tissue is called fracture callus. The fracture callus is replaced by woven bone a process similar to endochondral ossification.

In the long bone fractures, restoration of normal function takes three months in humans and in rats it is one month (Cullinane et. al., 2003) whereas remodelling takes a longer time. The twin characteristic of bone is continuous remodelling and regeneration of damaged tissue. Sometimes bone regeneration may fail due to poor blood supply, poor oxygen supply, inflammation, infection, fracture mobility or bone loss leading to the formation of cartilage (Pseudo arthrosis) or scar (Non-union).

1.15 BONE FORMATION

1.15.1 Endochondral Ossification

Endochondral Ossification is an essential process that occurs during fetal development of skeletal system through which bone tissue is created. During bone formation, condensation of cells occur leading to the formation of chondrocytes which are the primary cell type of cartilage (Meyer and Wiesmann 2006). These chondrocytes undergo cell division accompanied by the secretion of extracellular matrix. This process is known as interstitial growth. This matrix is rich in type II collagen and the proteoglycan aggrecan and also other transcription factors (Kronenberg 2003). As a result of chondrocytes proliferation and matrix production, the cartilage enlarges. Chondrocytes lying in the centre of cartilages stop proliferating and enlarges leading to hypertrophy. This changes the genetic signal and thereby synthesizes type X collagen and thereby initiates bone growth.

The first site of ossification occurs in the middle of diaphysis of shaft and this forms the primary centre of ossification. The perichondrium developed by the chondrocytes becomes the periosteum. The periosteum has a layer of

osteoprogenitor cells (Undifferentiated cells) which later turns into osteoblasts. To support the new bone, osteoblast secrete osteoid (oppositional growth) leading to the formation of bone collar.

Chondrocytes in the primary centre of ossification secretes alkaline phosphatase, an enzyme required for mineral deposition. As a result of this, calcification occurs in the matrix and the osteoprogenitor cells are stimulated to secrete osteoid on the calcified matrix. This forms the trabecula of bone. Osteoclasts breakdown the spongy bone to form the bone marrow (Meyer and Wiesmann 2006). In mammals, a secondary ossification centre appears in the epiphysis of long bones at the time of birth. Similar to the primary ossification centre, periosteal buds carry the blood vessels and mesenchyme. Epiphyseal plate is the cartilage present between the primary and secondary ossification centres which continue to form new cartilage. This cartilage is replaced by bone leading to the increase in the length of bone. Until the replacement of cartilage by bone happens in the plate, the growth of an individual continues.

1.15.2 Intramembranous Ossification

Intramembranous Ossification coupled with direct differentiation of mesenchymal stem cells leads to the formation of osteoblast which inturn replaces connective tissue membrane sheet with a bone tissue. This leads to the formation of flat bones (Frohlich et. al., 2008).

Both intramembranous and endochondral ossification are highly regulated by several molecules or pathways (Frohlich et. al., 2008). Both the ossification process shows vascularization as a common process. Vascularization occurs in intramembranous ossification during the transport of mesenchymal stem cells. In endochondral ossification, to replace the cartilage with bone and bone marrow, the hypertropic chondrocytes which act as a template for bone formation secretes angiogenic growth factors. This initiates the invasion of blood vessels. Angiogenesis plays a vital role in secreting the growth factors during bone formation which in turn regulates proliferation, differentiation and induces bone formation and bone resorption.

1.16 MATERIALS USED IN FRACTURE TREATMENT

Recently, the treatment of bone defects using bone graft substitutes with the principles of bone tissue engineering has gained importance. Traditionally bone defects are treated using autograft and allograft by a major process called transplantation.

1.16.1 Autografts

Autograft or autologous or autogenous bone graft is the tissue harvested from iliac crest or from the proximal tibia or from the distal femur and placed in the fracture or injury site (Zimmermann and Moghaddam 2011). Autologous grafts possess the important component of bone regeneration and features required for new bone growth namely osteoinductivity, osteogenicity and osteoconductivity. However, autologous grafts show limitations like morbidity at the donor site.

1.16.2 Allografts

Allograft is an alternative to autograft. Allografts taken from donor or cadavers too suffer with limitations such as infection, tissue matching, limited supply and disease transmission (Khan et. al., 2005).

1.16.3 Xenografts

The other type of graft called xenograft in which the tissues are harvested from living or non-living species. Xenograft like allografts also possess certain limitations and provide problems in future. Xenografts are used in filling up spaces in minor bone defects but still it cannot be an efficient bone substitute.

1.16.4 Metal Implants

Metallic implants are used in orthopaedic procedures like hip replacement therapy. In this therapy, the damaged hip is replaced by an artificial implant. The implant material used in this therapy depends on various factors like age and activity level of the patient and the surgeon's preference. The metals employed for this purpose includes titanium, stainless steel and cobalt chrome. For replacement surgeries, metal implants are classified into three types. They are (a) metal-on-metal implant, (b) metal and plastic implant and (c) metal and highly cross linked poly ethylene. These metal on metal implants and metal – plastic implants are used in hip joint replacement therapy in which both the ball and the socket are made up of metals. In metal-plastic implants a plastic spacer is placed in between the two metals.

1.16.5 Ceramics

The widely used material for biomedical applications is ceramics. Ceramics can replace the use of autografts if they satisfy the conditions such as restoring the function of the surrounding tissue, filling this space, physiologically interact with body fluids, stimulate osteogenesis and provide skeletal repair which could bear a permanent stress. Initially alumina and zirconia were employed for implantation. Ceramics due to its inert nature it have been used as crown, denture teeth, bridge, in reconstitutions of mandibular jaw, in augmenting the alveolar ridge and space filler in dentistry. Ceramics are mostly used as bone filing materials in further treatment of interposition of soft tissue, loss of bone, improper fixation, metabolic disturbances, infection and impairment of blood supply (Logeart-Avramoglou et. al., 2005).

1.16.6 Hydroxyapatite

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is one of the most widely used phosphate due to its similarity to natural bone mineral. It is well known for its biocompatibility and bio affinity which supports bone reconstruction. 50% of the bone weight is attributed by hydroxyapatite. In dental enamel and dentin, hydroxyapatite is the main mineral (Wang et. al., 2014). Theses crystals are also found in the pineal gland called corpora arenaceous of brain sand. Currently, HA has been used in various biomedical applications and bone tissue engineering. Due to its similarity with the inorganic part of bone, synthetic HA shows strong affinity with the host tissue thereby exhibiting good biocompatibility and osteoconductivity. HA consist of a hexagonal structure with a stoichiometric Ca/P ratio of 1.67. However, the mechanical properties and fragility is poor compared to the normal bone. Despite its poor mechanical strength, HA acts as a good bone substitute, due to its osteoconductivity, non-antigenic response in vivo and quick establishment for the new bone formation. Many researchers have worked on HA and used it in combination with other materials like gelatine, collagen, fibrin, alginate, chitosan, starch and casein (Ning et. al., 2015) used HA in combination with gelatine and chitin for healing the critical size defect in the proximal tibia of rabbits.

The synthesis of HA has been carried out by so many techniques like sol gel method, microwave method, co-precipitation, hydrothermal processing emulsion system, double step stirring technique and Sono chemical synthesis.

In tissue engineering, scaffold forms the template for cell interactions and form extracellular matrix to support the newly formed tissue. Since polymeric scaffolds lack certain properties like mechanical strength and osteoconductivity it is good to use composite scaffolds made up of bioactive ceramics in combination with biodegradable polymers. Composite scaffolds can be prepared in various combination of materials like bio glass, collagen/HA, poly DL-lactic acid/bio glass, poly (lactic-co-glycolic acid)/HA, etc. These scaffolds not only provide support for new tissue regeneration but also provide the biological signals. For this reason, scaffolds are always loaded with antibiotics, bone morphogenic proteins and growth factors. Some researchers used composite scaffolds as drug carriers in releasing the drugs proteins or genes.

1.16.7 Calcium Phosphates

In surgical treatments where bone substitutes are required, calcium phosphate ceramics are employed in various forms such as porous or dense powders and blocks. When a foreign material is placed in the body it leads to certain problems like toxicity, allergy, and inflammation. To overcome this, a bone substitute should be biocompatible, and should integrate with the bone tissue. These calcium phosphate ceramics exhibit excellent biocompatibility and hence they are used in bone tissue engineering. Solid bone substitutes made of calcium phosphate ceramics have been synthesized to mimic the natural bone. An ideal bone graft or bone substitute should contain porosity with a pore size of 100 μm in diameter, mechanical stability and osteoconductivity.

Metallic implants are coated with calcium phosphate ceramics by two methods. One is plasma spraying and the other is precipitation method. In plasma spraying method, solid calcium phosphate is injected into a plasma flame and which falls on the metal implant leaving a uniform coating. By this technique, metal and ceramic forms a tight bonding. In precipitation method, the metallic implant is dipped in a super saturated calcium phosphate solution. In this solution, biologically active compounds like antibiotics or proteins can be added. A uniform coating is given to

the metal implant along with the biologically active compounds which is more advantageous than the plasma spray method. In some studies, the implants were dipped in simulated body fluid (SBF), a solution whose inorganic ion composition is similar to human blood plasma to mimic the natural bone.

1.17 BASIL SEED

Ocimum basilicum L., commonly known as basil or sweet basil, is an annual spicy herb of the Labiatae family. The name basil is derived from the Greek word “Basileus” meaning “Royal” or “King” and it is often called “King of the herbs” due to its wide range of uses in medicine, cosmetics, and the pharmaceutical and food industries (Bilal et.al., 2012). Basil (*Ocimum basilicum* L.) is native plant of tropical regions. It belongs to genus *Ocimum* which comprises between 50 and 150 species of herbs and shrubs. It is one of endemic plant which is used not only as pharmaceutical plant but also used as culinary herb. Seeds of basil have been used as traditional medicine for treatment of dyspepsia, ulcer, diarrhoea and other illness. Many parts of Asia basil seeds are used to prepare traditional beverages (sharbat) and many ice desserts like falooda. This plant is originally from warm and tropical areas, such as India, Africa, and Southern Asia (Putievsky et al.,2005) and is specifically found in Pakistan and India, where it has been cultivated for around 5000 years. Today, it is found all over the world (Nadeem et al.,2020). *O. basilicum* is commercially cultivated in many warm temperate countries, including France, Hungary, Greece, and other southern European countries, as well in North and South America (Pushpangadan et al.,2012). Basil seeds are black in color and with oval shape and are good source of fibre. When seeds of basil are soaked in water they swell up and produce gelatinous mass because poly saccharide layer is present on outer epidermis wall of seed. This herb has been used in different ways from ancient times; the leaves can be used fresh or dried to add a distinctive flavor and aroma to foods. It is also used in the manufacture of beverages, liqueurs, vinegars, drinks, teas, and cheese, among others and the essential oils, which are extracted from the leaves and flowers, are used in the food, pharmaceutical, and cosmetic industries (Khaliq et al., 2017). The seeds are commonly added to beverages and ice cream and are also added whole or milled to bakery products as a source of dietary fibre for technological purposes. Moreover, the seeds are used to enrich fruit-based beverages for visual and functional purposes (Rezapour

et al.,2016). The seeds are high in dietary fibre and, thus, have huge potential as a functional ingredient (Naji-Tabasi et al.,2017). The mucilage extracted from basil seeds has been widely studied, and has emulsifying, foaming, thickening, stabilizing, viscosity, and gelling properties, among others. Basil seeds are not conventionally used as a food, despite its consumption stands out not only for its nutritious value but also for its significant health benefits, such as antidiabetic, antimicrobial, antioxidant, and anticancer activities (Gajendiran et al.,2016). Finally, the branches and soft woody stem can be added as a flavouring agent to different foods, the flowers are used in different dishes and beverages, and the roots are traditionally used in Indian medicine (Sharma et al.,2018). In general terms, basil leaves, flowers, seeds, branches, soft woody, and roots are used in both domestic and industrial applications, such as the food, pharmaceutical, and cosmetic industries (Kim et al.,2020). Basil has also been widely used in traditional medicine in the treatment of headaches, coughs, constipation, diarrhoea, warts, worms, and kidney problems. In addition, various pharmacological actions have been described, such as stomachic, antioxidant, antiviral, antimicrobial, analgesic, anti-inflammatory, antidiabetic, and anti-stress activities, and antipyretic diuretic and emmenagogue properties, among others.

CHAPTER 2

LITERATURE REVIEW

Anudurga Gajendiran et al (2016), presented a study in which basil seeds were used as the raw material for evaluation of their bioactive compounds. Basil seeds extract exhibited strong antibacterial activity against nine pathogenic bacteria. As per DPPH assay, the maximum free radical scavenging activity (73.85%) was shown by petroleum ether extract and the minimum activity (34.20%) was shown by methanol extract of basil seeds. Thus this study showed that basil seeds have good antimicrobial, antioxidant and anticancer activities

B.Akshal et al (2017), Synthesizing alternative bone graft materials are important in biomedical applications. Their morphology, mechanical properties and cell viability plays an important role in tissue engineering. Cell viability tests were conducted on the fabrication bio grafts. Thus the author showed that the cell viability increased in fabricated B-based bone grafts

E.Caroline Victoria et al (2002),BCP has been prepared using a precipitayion technique using 1.0M of $\text{Ca}(\text{OH})_2$ and 0.63M of H_3PO_4 .From XRD the HAP: analysis show the HPO_4 band in the as-dried sample.TG/DTG analysis show the formation of $\text{Beta-Ca}(\text{PO}_4)_2$ around 500 degree celcius and the subsequent decomposition of its pyrophosphate around 750 degree celcius to the formation of beta-TCP and calcium hydroxyapatite

Hector Calderon Bravo et al (2021), this study shows that Basil seeds contain high concentration of proteins, with all the essential amino acids except S-containing types and tryptophan; dietary fibre and linolenic fatty acids comprising the highest portions; minerals such as calcium, potassium and magnesium and phenolic compounds such as orientin etc. Their consumption is associated with several health benefits; such as the prevention of type-2 diabetes, cardio-protection, antioxidant and antimicrobial effects, anti-inflammatory, antiulcer, anticoagulant and antidepressant properties, among others

Jagadish H.Patel et al (2021),In this work chitosan hydroxyapatite composites were synthesized using the method of co precipitation for bone grafting. The samples were characterized using SEM, EDS and UTM. The composites showed

a good Ca:P ratio of 2.10, indicating a sufficient level of osteoconductive of the composites. Thus the author showed that the study can serve as a viable alternative to traditional bone grafting techniques, such as allograft and autografts.

Marc Bohner et al (2020), Beta TCP is synthetic, osteoconductive, osteoinductive and its resorption is cell mediator, thus making it one of the most potent bone graft substitutes. The aim of the present article is to provide a critical, but still rather comprehensive review of the current state of knowledge on Beta TCP with a strong focus on its synthesis and physio-chemical properties, and their link to the in vivo response.

Masooma Munir et al (2017), Basil seeds are not only as pharmaceutical plant but also for culinary purpose. The current study has been undertaken to develop a nutritious, healthy and value added drink. Proximate, mineral analysis, total polyphenol content and mineral analysis of basil seeds was conducted. Result revealed that basil seeds are not only good source of fibre and protein but they provide appreciable amount of minerals and phenolic compounds. This study showed that basil seeds could be supplemented in different food products for the preparation of value added, healthy and nutritious diet.

Mohamad Hesam Shahrajanian et al (2020), Basil is one of the most important crops with essential oils. Traditionally, it has been used in kidney problems, earache, menstrual irregularities, arthritis, treatment of colds and malaria. Basil has been shown positive effects against viral, fungal, bacterial and some infections. Thus this study showed the most important pharmacological uses of basil or anticancer activity, radio protective activity, antistress activity, antidiabetic activity, antipyretic activity, antiarthritic activity, antioxidant activity, as a prophylactic agent and in cardiovascular disease

Mythili Prakasam et al (2015), Dense and porous ceramics have been widely used for various biomedical applications. Current applications of bio ceramics include bone grafts, spinal fusion, bone repairs, bone filters, maxo facial reconstruction, etc. Amongst the various calcium phosphate compositions, hydroxyapatite which has a composition similar to human bone, has attracted wide interest. Prospective future applications, established along the aforesaid applications of hydroxyapatite, appear to be promising regarding bone bonding, advanced medical treatment

methods, improvement of the mechanical strength of artificial bone grafts and better in vitro/in vivo method.

Roselet P Cherian et al (2019), Basil seeds has a very high potential health supplement which is enriched with much more nutrients and beneficial chemicals. This study aimed to understand the health aspects of *Ocimum basilicum*. Thus the author studied that, basil seeds acts as an antioxidant which scavenges free radicals. It controls the blood pressure, boosts immunity and also it can be used to reduce weight. It can be used to cure modern health issues of human

R.Z.Legeros et al(2003).Currently Biphasic Calcium Phosphate is recommended for use as an alternative or additive to autogenous bone for orthopaedic and dental applications. It is available in the form of particulates, blocks, customized designs for specific applications and as an injectable bio material in a polymer carrier. BCP ceramic can be used also as grit-blasting abrasive for grit-blasting to modify implant substrate surfaces. Exploratory studies demonstrate the potential uses of BCP ceramic as scaffold for tissue engineering, drug delivery system and carrier of growth factors

S. Srividya et al (2018), this study focuses on fabricating a novel bone graft materials containing bio-polymers & phytochemicals which can replace the use of autogenous graft with high bio-compatibility and osteogenicity. Thus the author studied that bone graft materials should be bio compatible and osteogenic in nature

Srividya Seshadri et al (2014), In this study the bone graft was prepared using the extract of aloe vera, Beta tricalcium phosphate and chitosan to form a bone graft. The phytochemicals of aloe vera impact an anti-inflammatory and wound healing property to the bone graft in addition to its osteoconductivity. Hence the author showed that it can be used for fracture healing and other bio medical applications

Srividya Seshadri et al (2014), In this study the bone graft was prepared using Biphasic calcium phosphate and chitosan with the phytochemicals of *Coriandrum Sativum*. Thus the author has synthesized a bone graft with finer stability, ossification property, good mechanical and biological properties and it is showed that the bone graft is used for bio medical application sealing and other biomedical applications.

Xinggui Tian et al (2020), Orthopaedic implant associated infection constitutes one of the most devastating and challenging symptoms in the clinic. Implants without antimicrobial properties may become the harbourage for microbial colonization and biofilm formation, thus hindering normal bone regeneration processes. The above findings suggest antimicrobial Ag-THA may serve as a promising and easy to produce antimicrobial mineral for the development of antimicrobial orthopaedic composites implants to address the changeless in orthopaedic surgeries, especially where infection may changeling condition to treat.

CHAPTER 3

AIM AND SCOPE

- The study aims to fabricate a novel bone graft which will exhibit ossification and induce bone formation in the fracture site on a faster rate.
- To synthesize the hydroxyapatite
- To synthesize β tricalcium phosphate
- To prepare the *Ocimum basilicum* seed extract
- To fabricate the herbal bone graft and characterize it by physico chemical methods
- To characterize the Ob seed extract for its bio compatibility
- The fabricated herbal bone graft can be widely applied in fracture healing, dental implants and in other biomedical applications

CHAPTER 4

MATERIALS AND METHODS

95% Ethanol, ortho phosphoric acid, Calcium hydroxide, Diammonium hydrogen phosphate, Ammonia, and Calcium nitrate tetra hydrate were purchased from Sigma Aldrich.

4.1 PREPARATION OF THE PLANT EXTRACT

The seeds of *Ocimum basilicum* were collected and powdered in electric blender. The dried powdered was extracted using 95% ethanol in a Soxhlet apparatus. Using rotary evaporator the extract was concentrated, stored at 4 °C and the dried crude ethanolic extract was used for further study.



Fig 4.1: Basil Plant



Fig 4.2: Seed Powder

4.2 SYNTHESIS OF HYDROXYAPATITE

HAP was synthesized by using an aqueous solution of 0.5 M calcium hydroxide was prepared and to this 0.3 M ortho phosphoric acid was added drop by drop until the pH reaches 12.5. The mixture was kept in continuous stirring for 24 h. The resultant was then centrifuged at 6000 rpm for 15 min. The precipitate was collected, rinsed with double distilled water and then dried at 100 °C for 7 h

4.3 SYNTHESIS OF β -TRI CALCIUM PHOSPHATE (β -TCP)

β -TCP was synthesized by using an aqueous solution of diammonium hydrogen phosphate (25.76 g in 325 ml of double distilled water) was added to an aqueous solution of calcium nitrate tetra hydrate (69.675 g in 500 ml of double distilled water) under continuous stirring. To this, 16.5 ml of ammonia solution was added and stirred continuously for 2 h. The mixture was filtered, and the filtrate was rinsed with double distilled water (in order to remove the unreacted calcium and phosphate) and dried in the oven at 60 °C for 24 h. The flakes were then powdered and calcinated in the furnace at 850 °C for 12 h followed by cooling to obtain a white fluffy precipitate of single phase β -TCP

4.4 PREPARATION OF HERBAL BONE GRAFT

A 60:40 ratio of HAP and β -TCP was taken to prepare the BCP and to this 500 mg of Ob seed extract was added. To this mixture, Ob seed gel (1g of seed powder in 2 ml of distilled water) was added (Feng-Huei et al., 1999), casted into cylindrical graft and pelleted it. The graft was dried at 55 °C overnight and sealed in polythene covers and sterilized by Ethylene oxide.



Fig 4.3: Preparation of Bone Graft

4.5 INVITRO VIABILITY ASSAY

4.5.1 Preparation of test solutions

For MTT assay, serial two fold dilutions (6.25 – 100 µg) were prepared from this assay.

4.5.2 Cell lines and culture medium

Saos-2 cell line was procured from NCCS, stock cell was cultured in DMEM medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in an humidified atmosphere of 5% CO₂ at 37°C until confluent.

4.5.3 Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (1×10^4 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37°C for 24 h in 5% CO₂ atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula,

$$\% \text{ viability} = \text{Sample abs}/\text{Control abs} \times 100.$$

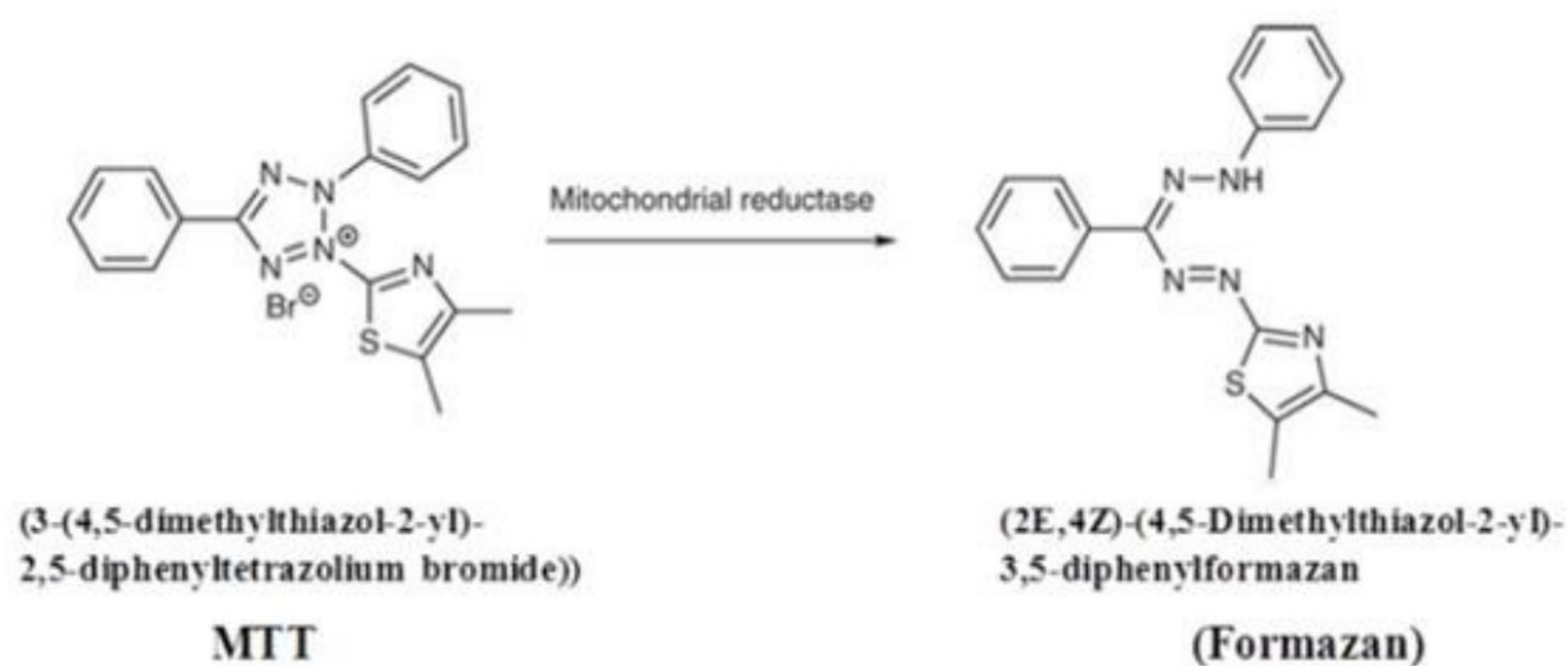


Fig 4.4: MTT

4.6 CHARACTERIZATION OF BONE GRAFT

The IR spectra of the prepared samples were read at 4000–400 cm⁻¹ using Nicolet Impact 400 FTIR spectrophotometer using KBr pellet containing 1–2 mg of the sample. XRD analysis of the sample was done using an analytical X'Pert PRO alpha-1 with a RTMS X'Celerator detector. It utilizes Ni-filtered Cu K α radiation over the 2 θ range of 20–80° with a scan rate of 2.4°/min and at a sampling interval of 0.002° at 40 mA and 45 kV. The surface morphology was analysed with a Zeiss Gemini Supra 55, SEM and EDX with Oxford instrument X-act. The copper disc was pasted with carbon tape and the sample was dispersed over the tape. The disc was coated with gold in ionization chamber before microscopic analysis. Seiko SSC 5200 H is used for analyzing the bone grafts for thermo-gravimetric analysis in a nitrogen atmosphere (80 ml/min) at a heating rate of 10°C/min. Primary weight loss of the graft which was due to the function of temperature was recorded using this study.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The FTIR spectra of HAP, β -TCP, BCP-Ob implant are shown in below figures. The phosphate bands were observed at 877.78 cm^{-1} , 963 cm^{-1} and 1023 cm^{-1} which attributes the anti-symmetric bending motion in HAP. The ν_1 phosphate band for of β TCP is observed at 941 cm^{-1} . The $-\text{OH}$ stretching bands of nano HAP shows the presence of hydroxyl groups at 3574 cm^{-1} and 3647 cm^{-1} . The 2800–3000 cm^{-1} wave number range is associated with the stretching modes of the C-H bonds of methyl groups ($-\text{CH}_3$), As it is found, the peak 1603 cm^{-1} may be assigned as $-\text{COO}-$ anti-symmetric and symmetric stretching bands of carboxylate salts, probably uranic acids. The second peak (1413 cm^{-1}) may be related to C-N stretching and primary amide. The third peak at 1023 cm^{-1} , which is seen at 900–1200 cm^{-1} range, represents various vibrations of C-O-C glycosidic and C-O-H bonds. In addition, these bands had an important effect on the gel forming and emulsifying abilities that can interact with water to have hydrogen bonds and make a proper gel and emulsion. It can be seen that the peak at 1656 cm^{-1} which are attributed to amid I band. 1509 cm^{-1} and 1561 cm^{-1} , which are indicating of amid II, C-N stretching and N-H bending.

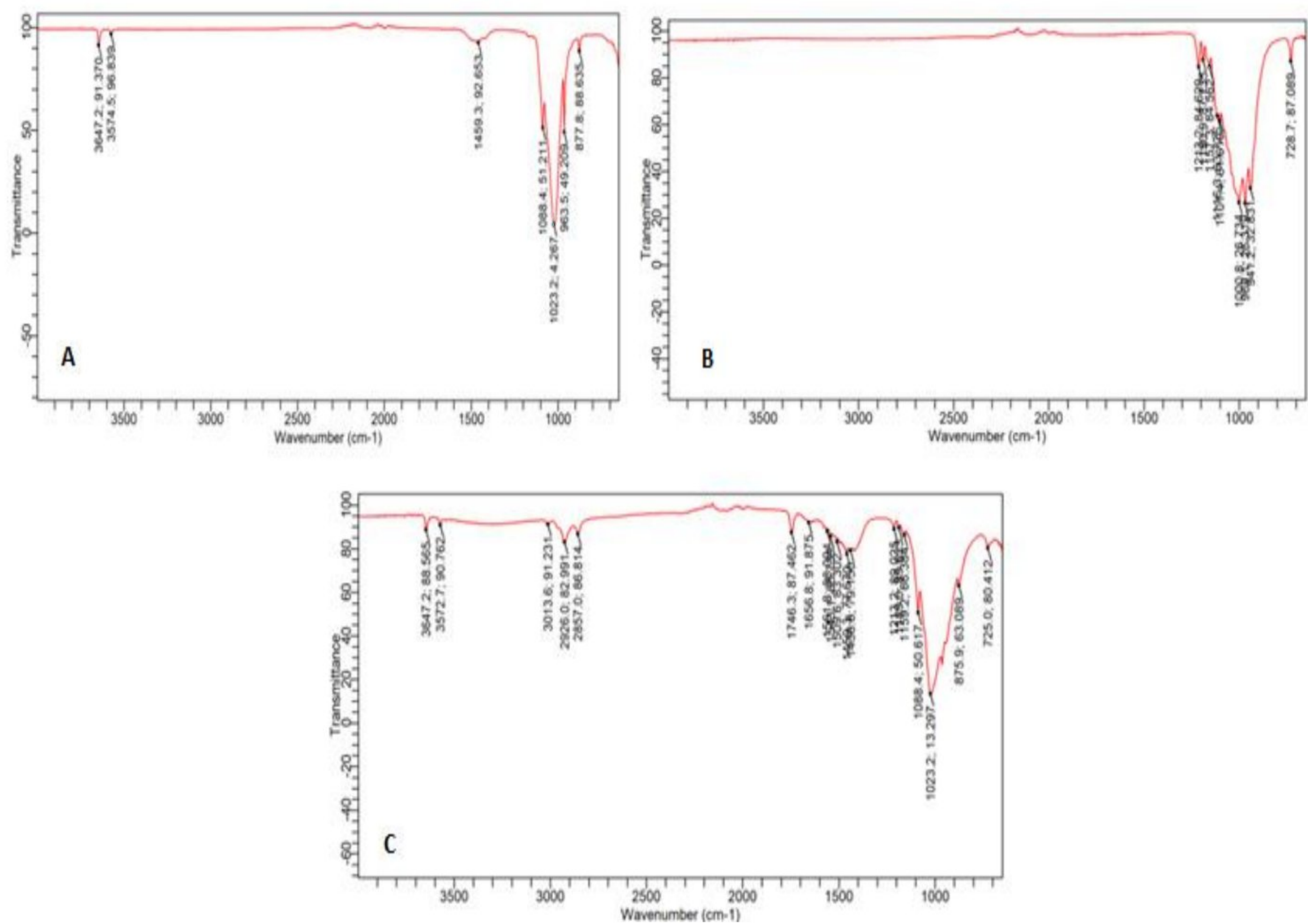


Fig 5.1: FTIR (A)Hydroxyapatite (B)Tricalcium Phosphate (C)Bone Graft

5.2 X-RAY DIFFRACTION (XRD)

Below Figures shows the X-ray diffraction (XRD) pattern of HAP, β -TCP, and BCP-Ob implant which suggests the presence of HAP and β -TCP in the bone graft. the addition of basil seed extract and seed powder decreases the crystallinity of HAP. The peaks at 26.18° , 28.45° and 29.25° indicates the reflection from 202, 002, and 210 crystal planes, respectively, thereby indicating the presence of Phyto nano HAP. The peaks at 32.13° , 33.22° , 47.18° and 53.48° indicates the reflection from 222, 112, 130 and 315 crystal planes, respectively thereby indicating the presence of samples show only characteristic peaks of HAP, TCP and these phases did not change into other phases during preparation of bone graft. This is important for achieving good mechanical and biological properties of produced bone graft (BCP-Ob).

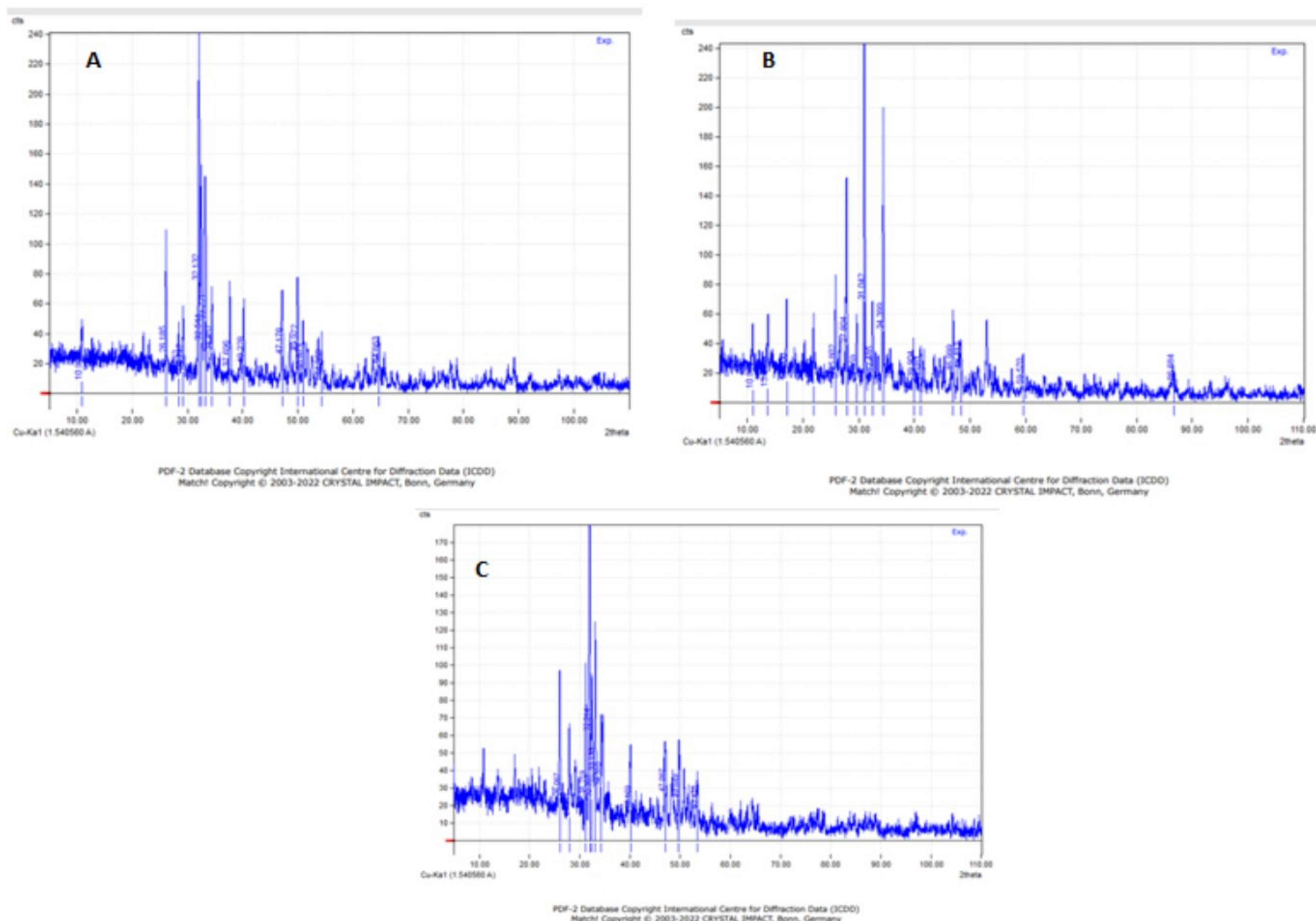


Fig 5.2: XRD (A)Hydroxyapatite (B)Tricalcium Phosphate (C)Bone Graft

5.3 SCANNING ELECTRON MICROSCOPE (SEM)

The SEM image of BCP-Ob bone graft is shown below. The basil seed powder forms the matrix for the graft on which the BCP is deposited. The incorporation of Ob gel is to create porosity through which the BCP and the OB extract reach the bone cells and induce ossification in the fracture site. An average porosity of $1.041\mu\text{m}^2$ is achieved by incorporating the Ocimum basilicum seed powder into the graft. The aggregation was seen due to the biphasic calcium phosphate deposition on the Ob matrix which with porosity favours the ossification property of BCP-Ob graft.

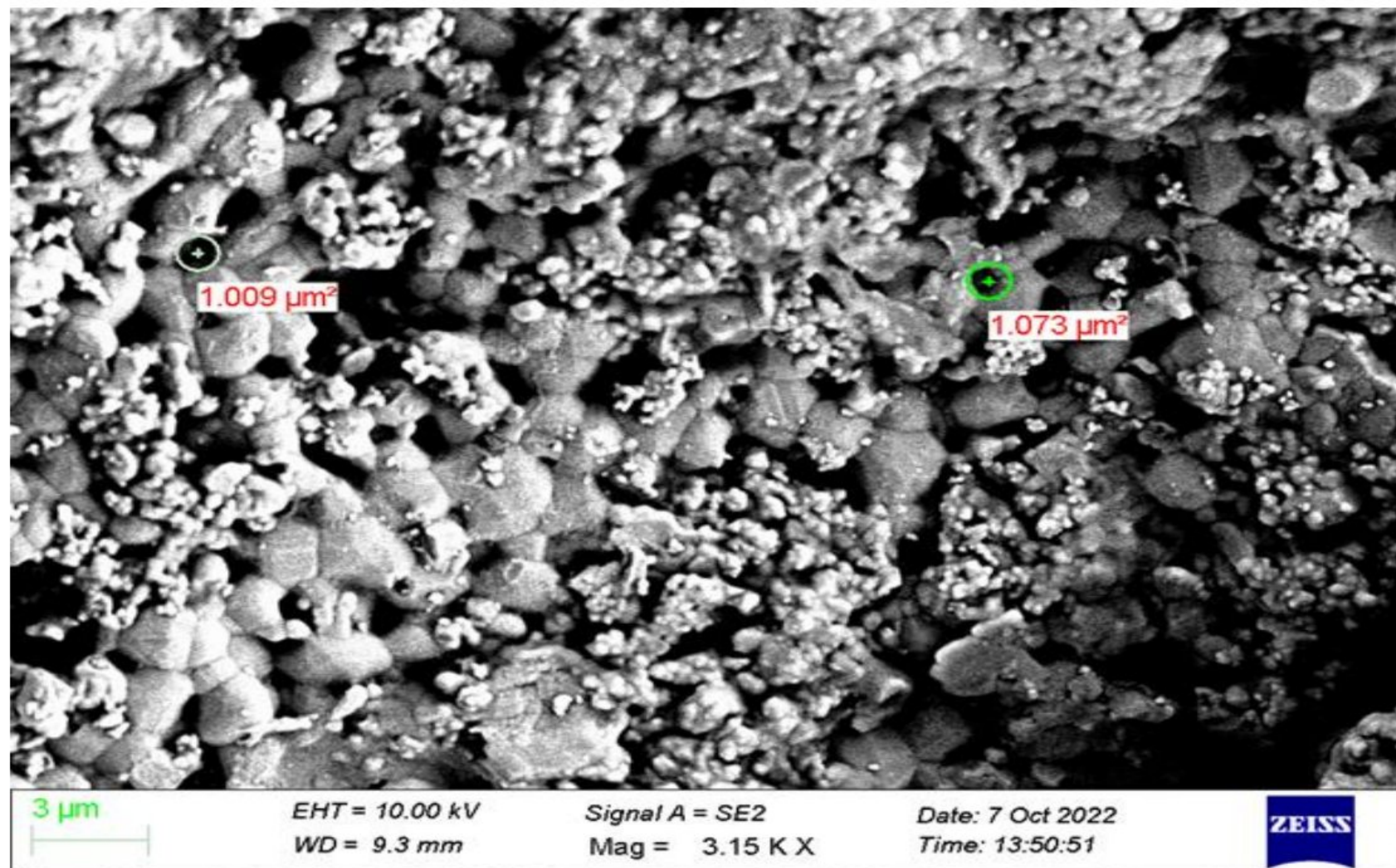


Fig 5.3: SEM Image of Bone Graft

5.4 THERMOGRAPHIC ANALYSIS (TGA)

Bone graft was subjected to thermal analysis using TGA and their results where shown in figure 5.4.

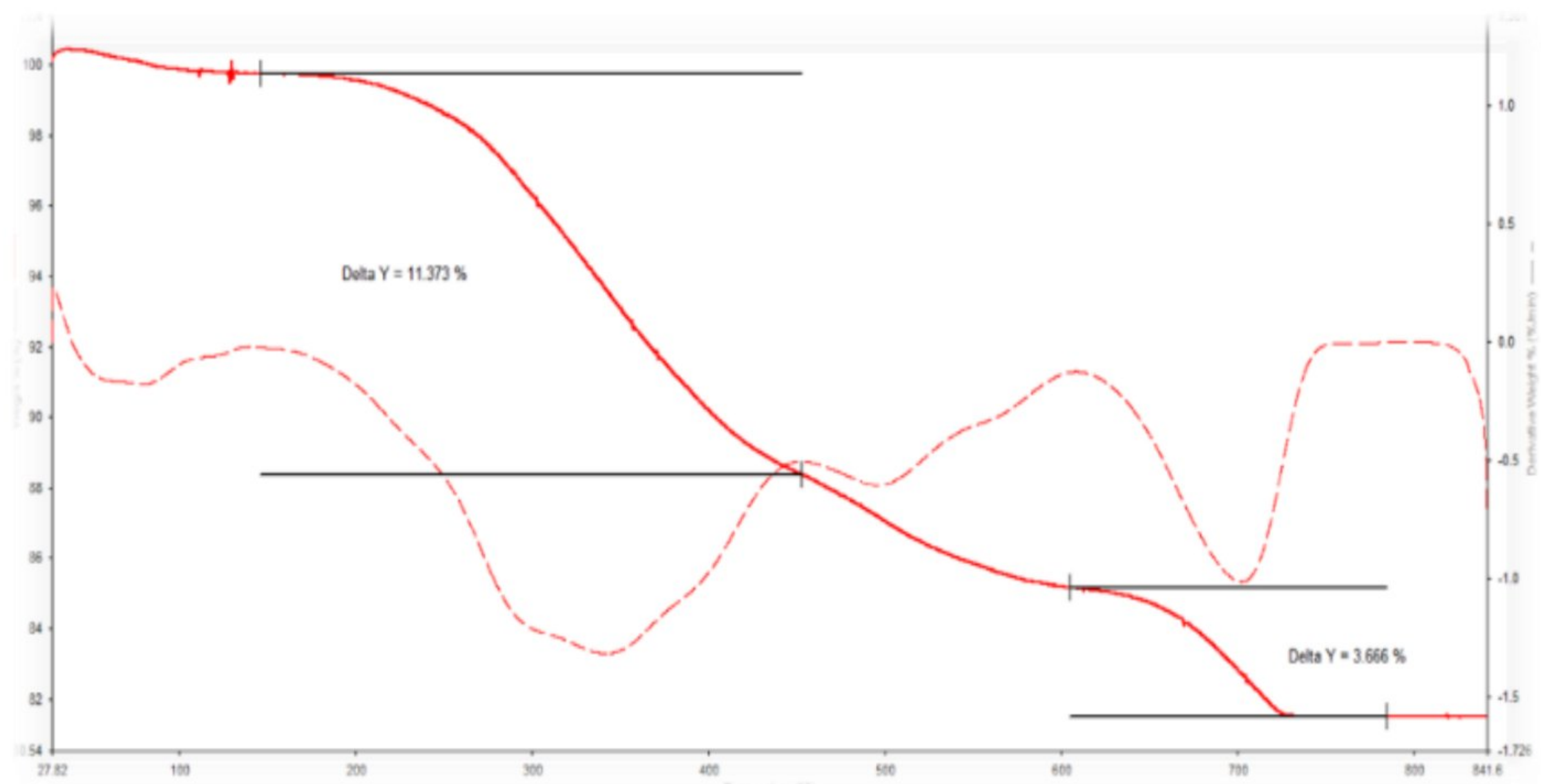


Fig 5. 4: TGA of Bone Graft

A single-step weight loss was observed at 110°C. This weight loss may be due to dehydration followed by a second weight loss occurring at 480°C which could be the decomposition of organic content of basil seeds. at the end of 740°C, the percentage of graft material retained was 3.66% which is the inorganic content of the graft. The results revealed the calcium and phosphorous content in the bone graft which helps in ossification of the graft in the fracture site.

5.5 GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

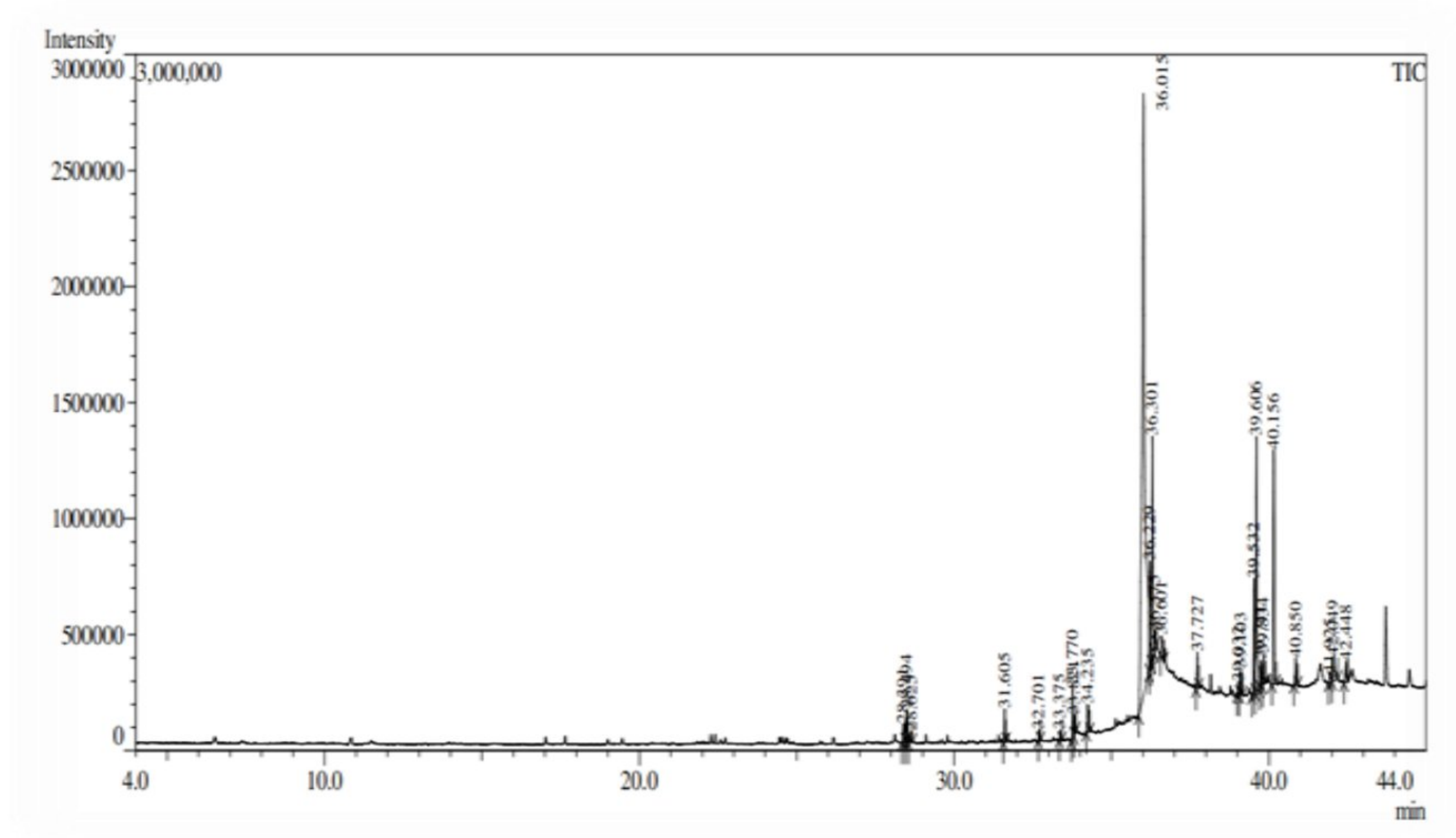


Fig 5.5: GCMS of Ob Extract

Table 5.1: Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	28.391	207424	0.65	1,2-BENZENEDICARBOXYLIC ACID
2	28.494	304275	0.96	1-Heptadecene
3	28.625	98343	0.31	Hen eicosane
4	31.605	273835	0.86	1-Nonadecene
5	32.701	74156	0.23	8-Octadecanone
6	33.375	78835	0.25	Hexadecanoic Acid Ethyl Ester
7	33.770	408472	1.29	Dibutyl phthalate
8	33.821	175116	0.55	N-Hexadecanoic acid
9	34.235	388589	1.22	HEXADECANOIC ACID,ETHYL ESTER
10	36.015	16777304	52.82	9,12,15-Octadecadienoate acid,(Z,Z,Z)-
11	36.229	1369098	4.31	n-Propyl 9,12-Octadecadienoate
12	36.301	2261366	7.12	ETHYL (9Z,12Z)-9,12-OCTADECADIENO
13	36.375	194369	0.61	NONAHEXACONTANOIC ACID,METHYL
14	36.601	252778	0.80	Octadecadienoate acid,ethyl ester
15	37.727	290992	0.92	Triarachine
16	39.032	105926	0.33	9,12-Octadecadienoate acid(Z,Z)-,2,3-dihydro
17	39.103	259863	3.82	ETHYL (9Z,12Z)-9,12-OCTADECADIENO
18	39.532	1168637	3.68	ETHYL (9Z,12Z)-9,12-OCTADECADIENO
19	39.606	3049272	9.60	Methyl 2-hydroxy-octadeca-9,12,15-trienoate
20	39.741	355539	1.12	Butyl 9,12,15-Octadecadienoate
21	39.834	302874	0.95	Triarachine
22	40.156	2071214	6.52	Phthalic acid,di(2-propylpentyl)ester
23	40.850	278906	0.88	Tetracosamethyl-cyclodocasiloxane
24	41.925	232845	0.73	n-Propyl 9,12-Octadecadienoate
25	42.049	514078	1.62	2,5-METHANO-1H-INDEN-7(4H)-ONE,H
26	42.448	269102	0.85	Tetracosamethyl-cyclodocasiloxane

In order to determine the compounds present in the extract of *O.basilicum* seeds, GCMS analysis was done. GCMS chromatogram of ethanolic extract of *Ob* seeds are presented in figure 5.5 and the bioactive compounds were listed in table 5.1. This analysis revealed that ethanolic extract of *Ob* seed contains different compounds. Some of them are known for their biological activity whereas, activity of few compounds remains unknown. From the *Ob* extract, Hexadecenoic acid, 1-Nonadecene, Octadecatrienoic acid, phthalic acid, Triarchies are reported to possess antimicrobial, anti-inflammatory and antioxidant activity (Kumar et al 2010 and Valvi et al 2014).

5.6 MTT ASSAY

The *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results.

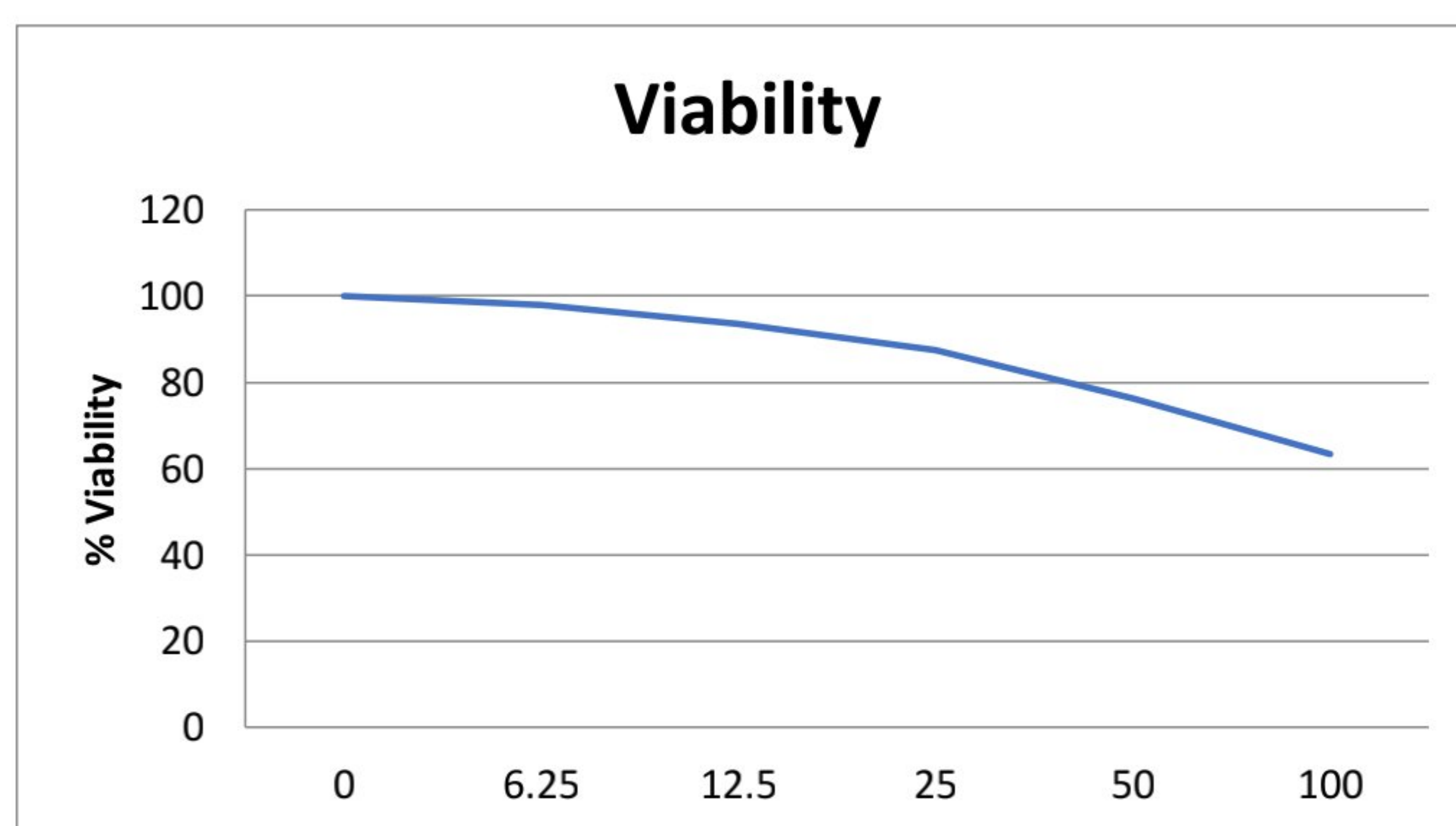


Fig 5.6: MTT viability assay of *Ocimum basilicum* seed extract

Table 5.2: MTT viability assay of Ocimum basilicum seed extract

CONCENTRATION in µg	MEAN	SD
0	100	0
6.26	97.85089182	0.402803351
12.5	93.48399364	0.581943136
25	87.51016319	0.667738572
50	76.12631306	0.351470861
100	63.3400564	0.139188042

The IC₅₀ value of the given **Ob extract** was found to be **>100 µg**.

The Ob extract subjected for cytotoxicity analysis in bone cell lines (SaOS2) showed very good biocompatibility till 25 ug/ml concentrations. Further IC50 is higher than 100 and hence considered as a safe material for cells and hence the extract was showed that it is highly biocompatible and can be used for invivo trials in the bone graft.

CHAPTER 6

CONCLUSION

In this study, a novel bone graft was prepared using BCP along with the phytochemicals of *Ocimum basilicum* (Ob) seed and used the seed powder as a matrix for holding the BCP in the graft. The anti-inflammatory, antimicrobial properties of basil seeds strengthens the acceptance of the graft in fracture site and also the porosity induced by the incorporation of Ob seed powder as a gel favours the osteoinduction and osteoconduction properties of BCP in the graft. Moreover, the material was subjected to various physio-chemical techniques to reveal the composition and the integrity of the graft. Further, this graft can be used for fracture healing and other biomedical applications, due its better osteoconductivity and biocompatibility. It can also be used as dental implants for treating periodontal defects

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