# Synthesis and Characterization of β-TCP and its Composite for Bone Tissue Regeneration


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# Certificate

This is to certify that work in this thesis has been carried out by Ms. Sumayya Noor and completed under my supervision in Functional Materials Laboratory, School of Chemical and Materials Engineering (SCME), National University of Sciences and Technology, H-12, Islamabad, Pakistan.

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# Dedication

Dedicated to my Family, especially my mother and my supervisor

Thank you for being there

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First, I praise and thank Allah Almighty, for His ultimate blessings throughout my work to complete the research successfully.

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# Abstract

Bone regeneration, which may be observed during typical fracture healing and is involved in continual remodeling throughout adult life, is a complicated, well-orchestrated physiological process of bone formation. The current study is based on the fabrication of gelatin and sodium alginate composite incorporated with β- TCP. Different amount of β-TCP was added to check the mechanical strength of the composite. The composite is highly biocompatible and biodegradable. To check the synthesis and fabrication of composite different characterizations like SEM, XRD, FTIR, Mechanical, and Water contact angle was performed. We have observed through these characterizations that the composite was successfully synthesized. The composite is highly biocompatible due to its hydrophilic nature which was observed by using contact angle. In this research we observed that β-TCP was added to limit where it increases the mechanical strength due to high surface to volume ratio. If the limit exceeds the mechanical strength start to decrease due to agglomeration.

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# List of Abbreviations

| | |
|---|---|
| DNA | Deoxyribonucleic acid |
| B | Beta |
| TCP | Tricalcium phosphate |
| FTIR | Fourier transform infrared spectroscopy |
| SEM | Scanning electron microscopy |
| BTE | Bone tissue engineering |
| XRD | x-ray diffraction |
| CA | Contact angle |
| T. E | Tissue engineering |
| MSC | Mesenchymal stem cells |
| HA | Hydroxy apatite |
| NP | Nanoparticles |
| PEG | Polyethylene glycol |
| PVA | Polyvinyl alcohol |
| PLA | Polylactic acid |
| HPMC | Hydroxypropylmethylcellulose |
| BMP | Bone morphogenic protein |
| PLGA | poly lactic-co-glycolic acid |
| ECM | extracellular matrix |
| UV | Ultraviolet |

# Chapter: 1

# Introduction

## 1.1 Nanoscience and technology

The Greek word nano means dwarf or something very small that represents a thousand million meters. Nanoscience and nanotechnology are distinguished from each other. Nanoscience is studied in the range of 1 to 100nm while nanotechnology is the utilization of technology in practical applications like devices etc. [1].

In 1959 Richard Fynmann said in a conference that “there is plenty of room at the bottom”. Then work on nanoscale made theirs on way in the market. Researchers and scientists have been able to develop integrated technology by combining knowledge from various fields of science thanks to nanotechnology. Nanotechnology and biotechnology create a multidisciplinary field known as nanobiotechnology to develop biomedical devices that incorporate biomolecular types of machinery like biomolecules and pharmaceutical ingredients into the material of choice. This functionalized material performs the function at the nanometric level. Targeted drug delivery, diagnostic imaging, therapeutic hydrogels, DNA chips and sensors, tissue engineering, implants, and other biomedical advances are examples of technological advances. Nanotechnology is integrated with multiple disciplines due to the wide range of opportunities available to exploit this field. Nanobiotechnology is concerned with developing new biomaterials, biomedical devices, and manufacturing/fabrication procedures to make the technology useful, accessible, and affordable A composite consist of two or more than two different materials and have different properties. The resulting material possesses properties not found in individual materials. Furthermore, when the materials are combined in a matrix, they must retain their distinct characteristics and boundaries. A composite consists of two phases: a continuous phase known as the matrix, which is in bulk, and a discontinuous phase known as the filler, which serves as reinforcement. In nanocomposite, reinforcement has 1 dimension in the nano range. Nanoparticles, nanorods, and nanosphere are examples. The properties of nanoparticles like thermal, mechanical, electrical, drug loading capacity,

water retention and so on can be improved because they have high subsurface-to-volume ratio [2].

Our research has focused on the synthesis and characterization of bio ceramic (β-TCP) and functionalized polymeric nanocomposite for biomedical application. The materials are easily available and affordable. Their modification and functionalization did not require special techniques and made the procedure cost-effective and reproducible.

## 1.2 Problem Statement

Diseases related to bone have been prevailing since decades, multiple strategies have been designed and being practiced for bone regeneration. Most of these existing procedures lead towards the re-surgery which does not convince patients to opt of better treatment methods.

## 1.3 Objectives

Following objectives were achieved to design a therapeutic, bio ceramic composite for bone regeneration

- Synthesis and characterization of β-TCP
- Synthesis and characterization of β-TCP based composite
- Effect of β-TCP on mechanical Properties of Composite
- Recording and interpretation of data through these techniques
- Overview of result and discussion of a comprehensive picture

# Chapter: 2

# Literature Review

## 2.1 Tissue Engineering

T.E is a technique by which lost tissues due to different pathological conditions either through the development of biological substitute can be restored, maintained, or improved. T.E general strategies can be divided into three categories

1: Firstly, implant or introduce tissue or cells into the organisms

2: Then delivering growth factors as a stimulating agent

3: and the last one is arrangement of cells with in or on the matrices [3]

There are many techniques used for T.E. T.E is an interdisciplinary approach to the improvement or replacement of biological tissue. The ideal scaffold must be osteoconductive, osteoinductive, bioactive, biodegradable, and biocompatible. Through the incorporation of physical interaction, tissue engineering strategies require interactions with cells and tissue.

Tissue engineering has two main approaches, one is scaffold-based, and the other is scaffold-free. In both these types, autologous cells are used. There are two other approaches introduced by engineers known as exogenous and endogenous cells.

### 2.1.1 Scaffold-Free Approaches:

Cells are directly administrated in this method. Cells can also be demonstrated by three-dimensional microsphere or cell sheet methods.

Growth factors are polypeptides that promote migration, differentiation proliferation and cell survival. Diseases of skeletal and cartilage can be treated by platelet rich growth factors. By using growth factors, we can increase the rate of regeneration.

### 2.1.2 Scaffold Base techniques:

In scaffold-based methods for cell differentiation, adhesion or viability both topological and biochemical aspects of scaffold should be reviewed [4].

## 2.2 Bone tissue engineering:

Bone is second most transplanted tissue, to treat bone abnormalities more than four million procedures are using annually with the help of bone graft or bone replacement [5]. Bone deficiency is one of the most well-known issues that dental surgery and periodontology practitioners deal with [6]. Throughout the world bone issue and condition has expanded. These days, bone repair is required even more for bone disorders including infections, tumours, and misfortune [7]. Bone has the capacity to regenerate itself, but some circumstances can hinder the regeneration of bone, so these issues are required to investigate. The scaffolds or grafts are required for large amounts of bone issues, so bone regeneration is going to gain attention in T.E [8].

BTE focus to maximize biological and engineering resources to enhance new bone regrowth [6]. In past few decades, BTE has among the most challenging methods for treating bone injuries. It often involves using full prostheses, scaffolds, hydrogels, and cells as biomaterials to repair bone abnormalities caused by fractures, osteoporosis, osteoarthritis, and neoplasms [9]. BTE has a several achievements in animal model using biomaterials and cells that ranges from adult osteoblast to bone marrow mesenchymal stem cells (MSC's). In fact, since the 1980s, MSC's potential for bone repair has been stressed [10]. Bone tissue engineering is a difficult and intricate method that starts with osteoprogenitor cells migrating and being recruited, proceeded by their matrix production, proliferation, remodeling of bone and differentiation [7]. Bone substitutes are required for diseases like cancer, infectious diseases, trauma, osteoporosis, fracture, and periodontitis. Therefore, BTE has emerged as a promising method for creating bone substitutes by fusing particular bone cells. These are following intentions of BTE

I. Describe status of bone regeneration by translational methods Fully comprehend the physical and chemical processes that govern cellular communications with biomaterials and the design of biomaterial devices that control cells,
II. Comparing different BTE techniques that involve adding osteogenic cells, genes, or proteins to the biological scaffold [11].

### 2.2.1 Clinical need in BTE:

There is very high regeneration capability of bone in young age and bone fracture can be healed easily. Due to lack of bone template large defects cannot be healed without medical assistance [10]. In elder patient the malunion and nonunion problems like fracture cannot heal naturally. The significant challenges in BTE are malunion and nonunion fractures [12]. Local immune abilities, early bone loss following surgery, osteomyelitis, osteostitis, and multiple surgical revisions are all factors in the non-healing of bone [13]. Acquired pathologies and congenital, such as failed arthroplasty, infections, neoplasm osteogenesis, imperfacta, failed arthroplasty including malformation, osteoarthritis, osteoporosis, trauma, osteomyelitis, spine arthrodesis, tumors and implant fixation presents a challenging problem for effective treatment [14].

The crucial size bone deficiency cannot be repaired during a patient's lifetime, necessitating replacement materials. In order to help, stabilize, and regenerate the bone, this procedure may involve the use of free fibula vascularized graft, metal work implantation, bone replacement materials and growth factors. Bone defects can be addressed by these grafts. These bone grafts are gaining attention due to their osteoconductive nature [5] [15]. Bone grafting demand is rising, and the range of biomaterials that can substitute bone transplants is expanding quickly. This increasing need is a result of the clinically necessary treatment of bone repair. The use of allografts, xenografts and autologous bone transplant is considered the gold standard in bone repair [16]. The iliac crest, ribs, and fibula are the sources of autografts for compact and trabecular bone. Osteoinductive, osteoconductive and osteogenic are properties of autograft. The optimal clinical outcome for autografts is that they contain

cells and growth factors that stimulate bone regeneration for high success rate. Because the do-nothings are the receivers themselves, they do not bring immunological problems, a risk of rejection, or a risk of disease transmission. The use of autografts is constrained by factors like pain, scarcity of autologous bone, the need of additional surgery, hematomas, morbidity at donor site and the possibility of infection and fracture. Particularly in children, the lack of supplies makes bone healing less feasible [16] [9].

Allogenic bone grafts come from a cadaver or another person. It has a variety of shapes and geometries. Allogenic bone transplants are preferable to autografts because they are more readily available and manageable. Allografts prevent the likelihood of donor site morbidity because they do not require the host to harvest the bone. Limitations of allografts include immunological rejection, disease transmission from donor to recipient, such as HIV, and lymphoma. Allografts require treatments such as acid washing, irradiation and freeze drying to prevent and eliminate any infection that could be present. During this procedure, mechanical and biological qualities are impacted [9] [17]. Xenograft can be obtained from cows and corals and are osteoconductive and osteoinductive. They are available very easily. They are cost-effective but they have drawbacks like poor immune response and have a risk of transmission of animal disease. Polymer, ceramic and metals are also used for bone grafting. The metals have good mechanical strength and integrity, but re-surgery infection and stiffness are its drawbacks [5].

## 2.3 Bone

Bone made up of minerals and is called connective tissue. In body bone performs many functions. Bone provides protection, locomotion, housing for bone merrow, structural integrity and store calcium and phosphate in body [12]. Bone continuously breaks down and rebuilds to fulfill these functions [18]. Bone is highly vascularized tissue provide mechanical stability to skeleton. Bone is a complex tissue. It is a heterogeneous composite material. It is composed 20-40 percent of organic constituent, 50-70 percent of inorganic constituent, 3 percent of lipid and 5-10 percent $H_2O$. HA is the mineral phase of bone and small amount of magnesium; acid phosphate and carbonate are present in it. Type I

collagen as well as proteoglycan and glycoprotein made the organic phase of bone. The rich tripeptide sequence in type I collagen promotes cell adhesion. Support, mechanical rigidity and load bearing is due to the inorganic phase, while elasticity and flexibility is due to organic phase. Lipids normally surround the cell's body and aid in cell activity and regulation. The water present in bone help in binding minerals, interact with collagen fibril and fill the pores [19]. Except sesamoid bones adult skeleton have 213 bones. Four types of bones are present known as long, short, irregular and flat bone

- Tarsals, carpals bones, patellae and sesamoids bones are based on short bones
- Ulnae, femurs, clavicles, metacarpals, phalanges, humeri and fibulae are based on long bones
- Mandible, ribs, scapulae, skull and sternum are based on flat bones
- Hyoid, sacrum, coccyx and vertebrae bones are irregular bones

Compact and cancellous bones make 80 percent and 20 percent of bone tissue. Cancellous bone is called trabecular bone while compact bone is known as cortical bone. Compact bone is dense. It is found on the surface of bone marrow. The structure of cancellous bone is honeycomb like structure. Cancellous bone is composed of trabecular rod and plate. It is present inside the bone marrow. Both bones are created in a lamellar pattern, which increases the strength of the lamellar bone. Periosteum and endosteum are the tissues present in compact bone. Outer surface of compact bone is based on periosteum and connected by collagenous fiber. The important function is played by them in repairing the fracture of bone. The inner surface of both compact and cancellous bone is composed of endosteum and connected with blood vessels, osteoblast and osteocytes. The ratio of compact to cancellous bone changes with different regions of bones and is higher in the endosteum than periosteum. Osteons are basic components of compact and cancellous bone. In compact bone osteon are known as harvesian system while in cancellous bone osteons are called packets [20]. In the extracellular matrix of bone two types of protein is present

1. Structural protein
2. Non collagenous protein

Collagen is a major while fibronectin is minor component of structural protein. Collagen is perfect for its function because amino acids' hydroxylation and glycosylation crosslink it.

Collagen is major component of structural protein while fibronectin is present in small quantity. Collagen is appropriate for its role because amino acids are crosslinked during the hydroxylation and glycosylation processes. Additionally, it promotes tissue elasticity, maintains the extracellular matrix, aids in or serves as a scaffold for the early mineral deposition, and binds various macromolecules. Fibronectin do the initial deposition of collagen fibrils. Cell signaling, matrix organization, metabolism and mineralization are done by noncollageneos protein [21].

Bone can undergo modelling and remodeling during its lifetime period. The technique by which bones change their overall structure in order to physiological considerations or mechanical pressures, gradually adapting the human skeleton to the forces it encounters is known as modeling. During bone modelling, there is little connection between bone production and resorption. Bone modeling is phase of upgrading. It helps in bone to make strength and hemostatic of minerals [19].

Osteoporosis is caused by imbalance between bone resorption and formation. It is age related problem. It decreases bone strength with age. Osteoporosis is Greek word; it means that bone decreases as the mass per unit volume of bone decreases [22]. Osteogenic cells with extremely particular functions and responsibilities are involved in the remodeling or repair of bone. These are osteoblast, osteoclast and cell of bone lining [14].

Bone forming cells are known as osteoblast. Osteoblast precursors are multipotent mesenchymal stem cells. The organic and inorganic secretion are released by osteoblast cells in the extracellular bone matrix. Along with osteopontin and osteocalcin, they are involved in the production of protein and the secretion of collagen matrix. Following active bone development, osteoblasts may transition into osteocytes, experience apoptosis, and become lining cells on the surface of the bone. On bone-forming surfaces, they produce fresh bone matrix [14]. 60-80% of osteoblast died through apoptosis. Bone resorption cells are known as osteoclasts. Mononuclear

cells of the monocyte-macrophage lineage are precursor of osteoclast. The precursor presents in bone marrow secretes a large amount of osteoclast. These are connected to bone matrix through integrin receptors. These have a ruffled border with multinucleated cell. They absorb and remove cells [14] [20]. Osteoclasts are also died through apoptosis. The most abundant cells are osteocytes and remain for decades in human bone. These help in supporting bone structure and metabolism. Osteocytes can transmit stress-based and strain-felt signals [20]. For normal hemostasis and functioning of skeleton they play important role. They connected through each other and also with surface of bone [14].

## 2.4 Composite:

Different physical characteristics of two or more different materials combined to form a single product is called composite. The final product has characteristics that the constituent materials do not have. Furthermore, when the components are integrated into a matrix, they should retain their unique characteristics and bounds. A composite comprises two phases: the matrix, which is continuous and constitutes the majority of the material, and the filler, which is discontinuous and serves as reinforcement.

### Nano Bio composite

A nanocomposite is a particular kind of composite in which at least one of the reinforcement's dimensions is in the nanoscale scale. Usually, these are nanoparticles (NP), nanorods, nanospheres or sheets, and so on. The qualities of composite materials, such as electrical, thermal, mechanical, drug loading capacity, water retention, and other properties, are greatly improved by nanoparticles due to their higher reactivity and surface to volume ratio. In most instances, the nanoparticles utilized in composites are inorganic. These can be ceramic NPs, silicates, clay particles, metallic NPs, or tiny pieces of clay. Nanobiocomposites are nanomaterials inspired by nature and made of biodegradable materials so, that they do not intoxicate the body when they come into contact with it. Typically, a biodegradable matrix is used, which can be either natural or manufactured and is made up of polymeric components like collagen, gelatin, chitosan, pectin, PEG, PVA, PLA, HPMC, and

others. On the other hand, the fillers are metal nanoparticles or ceramic (hydroxyapatite) (Ag, Au, Zn). As a result, biomedical technology that is compatible with human bodily systems has been developed. These biomimetic materials are either metabolically digested in the body without triggering an immune reaction against the device or they are physically and functionally compatible with the human body. Numerous applications, such as wound healing, tissue engineering, cartilage implants, gene therapy and medication delivery, have been created thus far [2].

## 2.5 Biopolymers

These are polymer obtained from natural sources. They are synthesized by chemically or biologically from organisms [23]. These are made from building blocks known as monomers by using environment friendly resources [24]. The biopolymer obtained from different sources are studied a lot due to their application in biomedical and pharmaceutical. Due to its diverse commercial positions, controllable physical behavior, and wide range of products, biopolymers have interest. They are cheap and renewable which makes them favorable in the field of biomedical applications and pharmaceutical.[23]. Biopolymer with another biopolymer, biodegradable polymer or nonbiodegradable and synthetic biopolymer combination can be possible. Additionally, biopolymers can be combined to create composite materials that combine a bio-polymer matrix with other reinforcing elements like mineral particles or natural fibres. To increase the mechanical properties and time of degradation they are combined with each other. On the basis of sources, it can be divided into two types

- Polymers that are extracted from nature are natural biopolymers
- Polymers that can be synthesized are synthetic polymer

Natural biopolymers include polysaccride and proteins while synthetic polymers include polylactic acid, polycaprolactone, and polyurethane etc. [25].

### 2.5.1 Biopolymer nanocomposite in bone tissue engineering

#### 2.5.1.1 Biopolymer nanocomposite

A solid polymer composite called a biopolymeric nanocomposite contains nanomaterials having at least one of its dimensions in nanoscopic range (1-100 nm)

[26] [27]. Nanosized materials are added in biopolymers to enhance its properties [24]. These nanomaterials can be nanocoating, nanotubes, nanoparticles, nanocrystals and nanofibers etc. This biopolymeric nanocomposite can be found as nanoparticle composites, nanofilaments, or nanolayer composites [27].

#### 2.5.1.2 Alginate nanocomposite:

Alginate is a marine algae-derived linear anionic copolymer made up of (1-4)-linked components of α-L-guluronate and β-D-mannuronate [28]. Alginate is natural polysaccride. Due to its biocompatible and biodegradable nature it is very efficiently used in BTE [29] [30]. It can be changed structurally and chemically for better use in regenerative medicine. Its porosity and viscosity allow for the prolonged inclusion of cells by the scaffold as well as their mobilization and inclusion. It is mechanically week, to increase the osteointegrative and osteoinductive nature and for making it powerful degradable polymer it combines with gelatin, hydroxyapatite and chitosan [31]. The high molecular weight alginate is used in BTE because they have good mechanical strength. The low molecular weight alginate is highly biodegradable. Stem cells alginate are used in BTE [29]. The oxygenation, vascularization, cell migration, adhesion, and growth that are biological processes necessary for the renewal of bone tissue are made possible by the alginate microparticle and microfiber scaffold [32]. Alginate-based biomaterials have a potential future for use in regeneration and repair of various organs and tissues, including cartilage bone and skin [30]. To improve the development with growth factor alginate is used to promote neovascularization in and around the skin [31]. Alginate has a high hydrophilicity and can produce stable hydrogels in the locality of several divalent cations, such as $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Ba^{2+}$ or $Sr^{2+}$. $Ca^{2+}$ is used to form alginate hydrogel by binding ionically with the alginate [33]. Alginate-based biomaterials contain distinctive physical, chemical, or biological properties that depict the environment of living cells. [30]. Alginate-PEG, alginate ceramic, alginate-collagen, Alginate-PLGA (poly lactic-co-glycolic acid), alginate-chitosan, alginate-gelatin, alginate-biosilica alginate-bioglass, alginate-bone morphogenetic protein-2, and peptides are some of the composite materials that have been studied. Alginate composites are more beneficial for bone tissue regeneration due to their increased biochemical importance in terms of biocompatibility, cell

proliferation, porosity, osteogenic differentiation, excellent mineralization and mechanical resistance. The mechanical performance of alginate is typically improved by the addition of synthetic polymers. Alginate's qualities and traits, such as its mechanical resistance, cell attraction, biodegradability, and gel characteristics can be changed chemically and physically [29]. The provision of chondrogenic or angiogenic development appeared to be particularly osteoinductive in hydrogels of (ALG/ECM) alginate/extracellular matrix, and this contributed to transform bone structure [34]. Bone morphogenic protein (BMP-2) was delivered using alginate to promote bone development and, in turn, enhance bone regeneration and scaffold vascularization [31]. Hybrid of chitosan and alginate show structural stabilization, high and fast vascularization, high mechanical resistance properties, and structural development of fresh bone [32]. To encapsulate cells, alginate has been utilized to control the relationship between the stiffness of the matrix and cell responses for bone regeneration [35]. As an alternative to bone transplant, chitosan-alginate with fucoidan and chitosan-alginate made using the freeze-drying method are reported. A successful fresh injectable BTE method uses arginine-glycin-aspartic acid (RGD)-alginate microspheres with osteogenic and endothelial tissues [32]. Cell adhesion is encouraged by the phase-separated alginate/hydroxyapatite scaffold, which has an 82 percent porosity at 40 °C, and this improves the mechanical properties. At ratio of 50/50 it contains high mechanical properties. The incorporation of this scaffold is done in rat containing osteosarcoma UMR106 and osteoblastic cell lines. 50/50 and 75/25 ratio of this scaffold found the better attachment [36]. Bone minerals with good mechanical properties are deposited by calcium phosphate-alginate hydrogel, which also demonstrates osteodifferentiation [37]. The healing and formation of bone can be done by using scaffolds consisting of gelatin and sodium alginate [32]. Preosteoblast spreading and proliferation and Osteogenic differentiation are facilitated by methacrylated alginate in combination with collagen. Mineralization and protein adsorption are improved by alginate-nano bioactive glass. Increased bone regeneration and osteoblast cell proliferation are caused by alginate-octa calcium phosphate. Rat bone is filled with collagen from alginate-HA. Rat MSCs demonstrates proliferation and cell survival in a sodium alginate-injectable calcium silicate

hydrogel with pore sizes of 50–200 nm, as well as possible ALP expression and angiogenesis. Proline-histidine-serine-arginine-asparagine (proline-histidine-serine-arginine-asparagine) and Alginate/RGD are ECM substitutes in bone repair [29]. The scaffold of sodium alginate (SA) nanocomposite with glucosamine-grafted hydroxyapatite nanoplate (gHAP) promotes osteoblast cell proliferation, adhesion, and differentiation [38]. The combination of 3D mesoporous bioactive glass with an alginate scaffold is intended to improve bone tissue engineering [29]. In non-union defects and massive bone defects, an electrospun nanofiber mesh tube with peptide-modified alginate hydrogel regenerates bone. Alginate hydrogel that contains nano-Hydroxyapatite (nano HA)/collagen particles exhibit high biodegradation. Biologically active cells are delivered by alginate scaffolds for bone repair. Alginate-HA composite likewise carries out the same function. Three-dimensional scaffolding of materials such as microcapsules, sponges, hydrogels, alginate fibers and foams is simple to process for applicable applications [29] [30].

#### 2.5.1.3 Chitosan nanocomposite

The exoskeletons of mollusks, insects cuticles, crustaceans, and fungal cell walls all include the abundant polysaccharide chitosan. Rouget discovered chitosan in 1859. It ranges of molecular weight is between 50-1000 kDa [39] [40] [41]. As like dissolves like chitosan is insoluble in concentrated and aqueous solution but when it continuously stirred it can be soluble in aqueous solutions like $CH_3COOH$, $HNO_3$, HCOOH, HCl and $H_3PO_4$ etc. [42] [43]. Chitin can be partially or fully deacetylated to produce chitosan. In essence, chitosan is deacetylated chitin. Because of the cationic nature of the chitosan scaffold, it is simple to switch out negative polymers like proteoglycans [43] [44]. It is copolymer and made up of 2-acetamide-2-deoxy-β-D-glucopyranose and 2-amino 2-deoxy-β-D-glucopyranose (glucosamine) [6] [40] [43]. Its amino and hydroxyl groups are modifiable, offering chemical diversity, improved solubility, and novel functional properties. Compared to chitin, this modification is simpler since the $NH_2$- group is located at the Carbon-2 position of the monomer ring [40] [45]. Due of its crystalline nature, chitosan promotes both strong intra- and intermolecular hydrogen bonds [41]. DD is unit of percentage of glucosamine, which is influenced by the degree of crystallinity (CD). This DD changes CH3COOH into

NH2- to transform chitin into chitosan. Chitosan that is commercialized has a degree of deacetylation of between sixty and ninety percent. DD is indirectly related to rate of degradation. Faster degradation occurs when the degree of deacetylation is low, between sixty-five and eighty two percent. The degradation is slower when the degree of deacetylation is high, between eighty-four and ninety percent. A large molecular weight will also result in a high viscosity, which provides resistance to flow and so encourages contact in tissues. pH values below 6 cause the free amino group to become protonated and soluble in weak acids. This protonation causes electrostatic repulsion and encourages the polymer to grow. The free amino group deprotonates and becomes insoluble at pH levels higher than 6.5. The swelling and breakdown process is lessened as the molecular weight increases [40] [41] [46]. The chitosan has hydrophilic, non-antigenic, bioactive, biodegradable, and antibacterial properties [6] [43]. Viral contamination, metal ions, and other contaminants have a significant impact on the viscosity, solubility, and biological activity of chitosan, among other features. Its limited mechanical strength and anti-microbial capability are shortcomings that could be remedied with chemical modification. Depolymerization of chitosan produces oligosaccharides with strong antimicrobial activity [47]. The cationic version of the amino group likewise exhibits this characteristic [40]. The antibacterial property is additionally enhanced by the addition of antibacterial metal ions, such as Cu, Zn, silver and nanophase [47]. In term of acid base characteristics, chitosan and its precursors are quite basic [40]Chitosanbreaks down when new tissues develop without releasing poisonous substances or triggering inflammatory responses [6]. Various goods can be made from chitosan by processing, including scaffolds for use in orthopedic applications and beads, fibres, films, gels, sponges, microparticles, and nanoparticles [47]. Adipic, glutaric and succinic acids are used to create chitosan hydrogels, which are then cross-linked with non-toxic substances like dicarboxylic and tricarboxylic acids to provide biocompatibility. Chitosan is a great material for biomedical applications since it has a high absorption capacity and swelling when fewer cross-linking agents are used [40]. A 3D network made of covalent and noncovalent bonds connects the hydrogel. For bone tissue engineering, scaffolds with pores between 100 and 200 μm are ideal [42]. The amount of cross-linking impacts

the mechanical strength, percentage of swelling, and pore size [40]. A porous scaffold made of chitosan can be created to multiply osteoblast and mesenchymal cells, causing mineralization and neovascularization. Chitosan's ability to absorb water facilitates the movement of nutrients and fluids out of the body. When cross-linking agents are used, chitosan, which is typically stiff and brittle, becomes more resilient and stretcher. The maximum and minimum osteoconductive and osteoinductive properties are present. Chitosan derivatives with high osteoconductivity include imidazole-chitosan, N-carboxyl butyl chitosan, and 6-oxylchitin. Chitosan is modified by copolymerization, phosphorylation, sulfonation, hydroxylation quaternization, and carboxylation in the field of BTE. This enhanced chitosan has better bone-regeneration abilities [47]. In bone tissue engineering, chitosan encourages osteoblasts to proliferate and deposit a mineral-rich matrix. Because it is so simple to inject, chitosan is frequently utilized in conjunction with bone cement during surgery. It aids in bone growth by encouraging osteogenic cell adhesion. It can serve as a scaffolding material in the engineering of cartilage due to its similarity to different glycosaminoglycans. Due to its higher tensile strength, chitosan made from alginate is employed as a load-bearing template for cartilage tissue regeneration. CS would support osteogenic cells in spinal fusion for spine tissue engineering because of its macroporous structure and predictable rate of breakdown [48]. Nano TiO2 doped chitosan scaffold produced by freeze drying has outstanding mechanical properties, high porosity, and strong biocompatibility. These characteristics increase the adhesion, proliferation, and mineralization of bone cells [44]. Since Chitosan enhances the properties of inflammatory cells, wound healing increases [45]. Chitosan and its derivatives are used in template to encourage the tissue regeneration of bone and cartilage [49]. Chitosan is bioabsorbable, making it a great matrix for nonviral gene therapy [48]. A nanocomposite made of CS, hydroxyapatite, zirconia and organically modified montmorillonite was created by Bhowmick et al. The osteoblastic cell proliferation and mechanical properties are enhanced by zirconia nanoparticles. High tensile property, swelling, erythrocyte compatibility and Strong antibacterial activity were produced by using 5% OMMT and 5% HAP-ZrO2 nanocomposite in 90% CTS [50]. A Chitosan/nanohydroxyapatite/nano zirconia

scaffold was created by Balagangadharan et al. and combined with the bioactive microRNA miR-590-5p. This scaffold boosts the osteoconductive property and promotes osteoblast development [51]. A chitin-chitosan scaffold that contains nano ZrO2 stimulates osteogenesis, has good swelling properties, and has a regulated rate of disintegration. Nanostructured cubic zirconia films improve the adhesion and osteoblast cell development [52]. So, it is suitable material for BTE.

#### 2.5.1.4 Collagen nanocomposites

Collagen is natural biopolymer. It is a ubiquitous protein that make the one third part of whole-body protein [53]. It originates from rat tail, porcine, bovine or rabbit bone [54] [55] [56]. These are mostly present in skin, tendon, ligament and bone are present in the extracellular matrix [56] [57]. Since collagen is the main component of the fibrous layer that lines the periosteum, collagen membranes can help and enhance the proliferation of periosteal cells in humans. It is essential for cell motility, adhesion, proliferation, and differentiation [58]. In vertebrates there are 28 type of collagen is present [53] [59]. The most common type of collagen among them, implanted in bone, is type I, whereas type II and type III collagen are found in cartilage [53] [60]. Collagen has four types of structure known as primary, secondary, tertiary and quaternary. It is highly hydrophilic. The repetitive tripeptide sequence of amino acids (Gly-a-b)n makes the primary structure of collagen. In this amino acid a is often proline and make 20% of the amino acid. The b is mostly hydroxy proline and contributes 50% of the whole amino acid. Tripeptide repeating unit caused secondary structure and they are mostly α-chains. Collagen molecules crosslink covalently with one another or with other ECM molecules to form this structure. Triple helixes make the tertiary structure of protein in which a and b form the outer covering of helix while glycine make the central axis. Collagen fibrils are a quaternary structure where molecules self-assemble in a supramolecular manner. For the quaternary structure to be preserved, collagen molecules are crosslinked. Various types of collagens can be effectively created, including sponges, composite, membranes, films, powder, blends, fibers, gels, particles and solution [53]. Due to gelling ability, excellent susceptibility and good swelling for enzymatic degradation makes the collagen hydrogels very suitable for bone tissue engineering [53]. Collagen is highly used scaffold in BTE due

to its highly biocompatible and degradable nature [59]. Tissue regeneration and Cell migration are increase by collagen scaffold. To increase the functionality of tissue engineering it is necessary to crosslink the collagen scaffold. The crosslinking can be accomplished by using chemical agents or physical treatment. Physical treatment includes UV irradiation, gamma irradiation, microwave irradiation and dehydrothermal treatment. Chemical agents like glutaraldehyde and carbodiimides are often used [53]. Phosphatidylserine and collagen combine to create a nano bioactive glass composite that aids in tissue remodeling and binds to calcium ions due to its high affinity [54]. Osteogenesis and angiogenesis are demonstrated by collagen with bioactive glass, which makes it potentially useful for wound healing. After being implanted, bioactive glass-coated collagen disk show high porosity [61]. Angiogenesis and osteogenesis are stimulated by collagen that contains hydroxyapatite (HA) microparticles. The bioactivity and mineralization are enhanced when the same template is made using the freeze-drying technique. Osteoblast proliferation, angiogenesis, and vascularization are made easier by a composite of collagen with bioactive glass microparticles with cobalt and glycosaminoglycan (GAG) [54]. Bioactive glass with a collagen template displays better mineralization in a simulated bodily fluid [56]. Collagen that has been mixed with hydroxyapatite nanoparticles created using the suspension method encourages greater resorbability and mechanical properties [62]. Collagen's hydroxyapatite enhances the surface area of biopolymer, influencing cell adherence [59]. The presence of collagen in the CS molecule produces a rigid matrix that promotes cell proliferation. Due to their exceptional biocompatibility and cell affinity, in directed bone growth, collagen membranes are used. It is restricted to the transmission of disease from animal to human. It has weak tensile strength, and an uneven rate of degradation relative to tissue regeneration. For bone regeneration, collagen composites have features including bioactivity, osteoconductivity, and biocompatibility [53]. By mimicking the elements that help bones develop, collagen provides an environment that is conducive to bone production. The weak tensile properties of collagen template can be enhanced by crosslinking them or even mixing them with materials like calcium phosphates,

silica, bioactive glass, etc. Biocompatibility is decreased by this process [53] [61] [62]. Scaffolds with collagen microparticles promote osteoblast development.

Both collagen fibrils and collagen molecules, can be used to make collagen. Collagen is often extracted and purified via centrifugation, alkali treatment, precipitation, and acid treatment. The purifying procedure can reduce or eliminate the cytotoxicity of collagen. Since collagen is naturally hydrophilic, it instantly expands after implantation. Collagen is therefore treated with different ceramics or polymers to change its physicochemical properties. In order to overcome natural collagen's drawbacks, such as the inflammatory reaction, insufficient bioactivity and risk of infection etc., synthetic collagen is created to replicate natural collagen in the extracellular matrix [53]. Because collagen has a high degree of polymorphism and may take many different shapes, it typically forms a structural network in tissues.

Collagens mechanical strength, which serves as a scaffold for calcium carbonate and calcium phosphate accumulation in bone, is a key property that makes it a highly effective material for BTE [61]. It is hemostatic and has low immunogenicity and antigenicity [56] [63]. Collagen membrane has the potential to cling to cells and can stimulate fibroblast cells' DNA production [63]. Additionally, they have the ability to form a direct link with host bone and supply calcium for bone rebuilding [58]. Osteocalcin gene expression and In vitro cell proliferation are improved by collagen/alginate composite. Mesenchymal stem cell osteogenic differentiation is encouraged by the hydrogel of collagen and agarose [64]. Collagen and photopolymerized chitosan promote bone healing. Collagen and methacrylated glycol chitosan enhance bone marrow stromal cell differentiation and proliferation [65].

#### 2.5.1.5 Fibrin nanocomposite

Because of its properties like biodegradability and biocompatibility fibrin used as natural scaffold [66]. It is present in nature. It is produced by polymerization of fibrinogen enzyme. Fibrin is obtained commercially. Fibrin is the best choice for T.E applications, offering a number of advantages over other biomaterials [67]. A vital component of hemostasis, fibrin is made up of thrombin and a fibrinogen activity that contributes calcium [68]. Fibrin's function is controlled by varying the concentration

of its precursors, such as thrombin and fibrinogen, and by adjusting the polymerization variables, such as ion intensity and pH, which is an important feature for the design of the template [67]. The composite scaffolds made of fibrin that have been studied are non-toxic, biodegradable, and help cells grow and mature both in vitro and in vivo [69]. Fibrin is also used as an injectable scaffold. In association with the suitable cell sources fibrin act as a structural scaffold bone, cartilage, liver and tissue cells. In the development of connective and structural tissues the fibrin also plays an important role [68]. There are various cells and protein interaction sites are present in fibrin. Extracellular matrix (ECM) ligand concentration and growth factors are two biochemical indicators in fibrins that are affected by changes in polymerization parameters. These can affect the expression of growth factors and the properties of cell development, proliferation, and differentiation. Cell behaviour can also be influenced by physical factors like matrix stiffness. Fibrin is therefore allowed to serve as a bioactive matrix that is suitable for cell functionality, supply, and assistance [67]. It has the potential to be used in the creation of BTE products. Since fibrin is a natural template that aids wound healing by cell attachment, promoting angiogenesis and proliferation, it can boost the environment for bone regeneration [70]. Perka et al. created fibrin crystals with a diameter of under 3 mm that could encapsulate cells. The structure was inserted into aberrant rabbit ulnae. Fibrin bead communities' examination revealed a greater bone cure in cell encapsulation after 28 days. For twenty-eight days, Nair et al. employed HA cylinders coated with silica and fibrin-coated bioactive materials. Morphology, cell cycle and analysis of cell viability has demonstrated that significantly enhance cellular function [71]. It is frequently utilized to produce new bone cells or to fill bone with coagulation in order to treat subcritical defects since it has strong adhesive properties on biological surfaces. Under fibrin coatings, the poly (lactic acid) (PLLA)-based scaffolds increased the adhesive properties of the scaffolds, promoting rapid tissue development. Hydrogel scaffolds based on fibrin were frequently utilized to construct cartilage and bone formation [72]. Plasma from autologous donors can be used to develop a fibrin-based adhesive. Without any graft material, the fibrin glue implant showed significantly greater osseointegration. The results of this study demonstrate that platelet-rich fibrin glue

might serve as a suitable scaffold to enhance implant healing and bone incorporation [73]. Tricalcium phosphate (TCP) and fibrin glue mixed with autologous bone fragments successfully repair dog cranial bone [73]. In rabbit calvarial failures, the fibrin adhesive mixed with autologous bone similarly had equivalent results, according to Lappalainen et al. The negative influence has been explained by the possibility that fibrin limits the vessel's access into the transplanted bone and inhibits the production of natural fibrin. In contrast, heterologous fibrin impaired bone healing, according to Bosch et al., homologous fibrin stimulated the growth of capillary arteries and connective tissue cells as a result of the local immune response, resulting in the rapid re-formation of bone.

Bone healing is aided by the addition of osteogenic cells to a fibrin matrix. Among the effective biopolymers for BTE is fibrin. The combination of remarkable biodegradability, biocompatibility, inherent bioactivity, and many other specific properties makes fibrin an interesting biomaterial for bone tissue engineering [71].

#### 2.5.1.6 Gelatin nanocomposites

Gelatin is a biopolymer occurs in nature and it is a protein that can easily be soluble in water that is created by the acidic or alkaline hydrolysis of insoluble animal collagen found in tendons, skin and bones [56] [74] [75] [76]. Gelatin is of 2 types. One is type B gelatin while other is type A gelatin. Gelatin type A is created through the acid hydrolysis of bovine collagen or hog skin using HCl and H2SO4. Between eight and nine gelatin type A has an isoelectric point. Animal skin or porcine collagen is used in the alkaline hydrolytic procedure to create gelatin B. Between four and five gelatin type B has an isoelectric point. In essence, gelatin is a collagen derivative that has undergone partial denature. Gelatin has no flavour and no aroma, and it is a pale-yellow color. There are 19 amino acids in it, have molecular weight from fifteen thousand to four lac Da. For every 1000 residues, there are 112 alanine,132 proline, 330 glycines and 93 hydroxyprolines. Gelatin's triple helix shape results from the repeating sequence (Glycine-Y-Proline), where Y is often one of the amino acids lysine, arginine, methionine, or valine [56] [74] [75] [76] [77]. Gelatin is amphoteric in nature because it has functional groups of both acidic and alkaline medium. Low

antigenicity, non-immunogenicity, and strong biocompatibility and biodegradability are its characteristics [76] [77] [78]. They are inexpensive, and changing the functional groupings is simple [74] [76]. The tensile strength of the electrospun PVA (poly vinyl alcohol)-Gelatin nanofiber membrane is increased by the addition of biphasic calcium phosphate [76]. Gelatin-nano Hydroxyapatite with PLGA added enhanced migration differentiation, cell adhesion, and osteogenic differentiation [56]. Gelatin-polycaprolactone nanofibers are an effective scaffold for bone marrow stromal cell culture that promote proliferation and cell adhesion [76] [78]. For four weeks, PLLA in gelatin-nano hydroxyapatite stimulated bone regeneration in rabbits and improved the mechanical properties [56]. Cell adhesion and growth are supported by PLLA-gelatin scaffold. A PCL-gelatin scaffold used in BTE encourages proliferation of human adipose-derived stem cells [74]. MSC's are encouraged to differentiate by the presence of graphene nano-flakes in gelatin hydroxyapatite [56]. In a rat calvaria model, gelatin-HA microparticles of 5–10 m in size stimulated bone regeneration. Gelatin composites containing TCP, BTCP, HA, and octacalcium phosphate were proposed as appropriate structures for use in BTE. Gelatin template in the rabbit ulnar critical size model, rat tibial bone defect model, rat distal femoral condyle defect model, and x-ray irradiated designs, have recently been evaluated in this context [75]. The hydroxyapatite-chitosan-gelatin composite membrane boosted the production of osteogenic differentiation of human MSC's and extracellular matrix enzymes in the absence of pharmacological stimulation. An outstanding cell bonding and penetration was achieved using a porous nano-TCP-gelatin porous matrix template that provided a platform for transporting nutrients and re-moving waste. Mesenchymal stem cells in rats have increased proliferation and osteogenic differentiation thanks to a biomimetic composite gelatin/-TCP, composite fibers. A new structure of the gelatin-bioactive glass-silver nanoparticle was created by Yazdimamaghani et al. Viability and gram-negative antibacterial activity of human mesenchymal stem cells E. coli and gram-positive bacteria The evaluation of Staphylococcus aureus led to the suggestion that gelatin-bioactive glass-nanosilver scaffolds be used as antibacterial scaffolds. Gelatin has been applied to scaffolds made of 45S5 Bioglass and combined with the antibiotic tetracycline hydrochloride. This polymer-covered scaffold had controlled drug

release, served as a versatile template and improved mechanical properties, for the production of bone tissues [56]. The cross-linking of the gelatin strands is crucial in medical applications, particularly tissue engineering, for improving mechanical and thermal strength under physiological conditions. Crosslinking gelatin hydrogels can be accomplished by chemical or physical methods. Formaldehyde, glutaraldehyde, poly-epoxide, dimethylsuberimidate, tannic acid, and acyl azide were just a few of the crosslinkers utilized to join gelatin together in order to enhance its incorporation into living tissues and improve the tensile strength of the composites. OPCs which are present in many organic products,nuts, seed, vegetables, and plant barks, are marginally less cytotoxic crosslinkers. As a scaffold for filling bone deficiencies, genipin interconnected gelatin with TCP ceramic particles can be employed. Bone replacement uses gelatin with TCP and oligomeric proanthocyanidins as cross-linking agents. A scaffold that is osteoconductive and biocompatible can be made using tricalcium phosphate and gelatin. The chemical and physical structures of natural bone extra cellular matrix were replicated using the thermally induced phase separation method in nanofibrous gelatin-apatite composite. The template showed exceptional mechanical strength and biocompatibility, and the addition of apatite enhanced osteogenic differentiation. Increased alkaline phosphatase activation, osteogenic gene expression development and cell attachment in mouse bone marrow MSC’s were all brought on by the addition of TCP to the gelatin nanofibers [75] [77] [78]. Osteogenesis is promoted by the nanofibrous gelatin-tricalcium phosphate composite in bone marrow stem cells. The gelatin methacrylamide hydrogel scaffold has a substantial impact on BTE because it enhances rat adipose-derived stem cells' osteogenic differentiation, proliferation, and adhesion to the scaffold. As a result, it can serve a variety of purposes in the field of bone tissue engineering. Large bone defects are typically treated using gelatin, a pure polymer and nanofibrous scaffold. The ability of gelatin to integrate with both synthetic and natural polymers favours a high bioaffinity and biomechanical making it a promising material for T.E scaffolds [79] [75] [74].

### 2.5.2 Applications of nanocomposites

Nanocomposites have a wide range of applications, which is growing rapidly. . In the next five to ten years, the following major areas are expected to be covered by the predicted global output, which is expected to exceed 600,000 tonnes.

- UV protection gel
- Drug delivery system
- Anti- corrosion barrier coating
- Lubricant and scratch free paints
- New abrasion resists materials
- New fire-retardant materials

Nanocomposite is quite popular in many automotive and general/industrial applications due to improvements in mechanical properties. These possibilities include door handles, the ability to be used as mirror housing on various engine covers, intake manifolds, vehicle types, and timing belt covers. Currently being investigated are more widespread uses including blades vacuum cleaner impellers, mower hoods, power tool housings, and covers for portable electronic devices like superior strength fibers, films and mobile phones [80].

## 2.6 Gelatin

Gelatin is a hydrophilic biopolymer extracted by partial or full hydrolysis of collagen. It is cheaper and highly available [81]. It is nonimmunogenic, noncarcinogenic, and has low antigenicity [82]. Upon cooling below gelation temperature, the helical structure of collagen can be recovered [83]. Upon increasing the temperature gelatin can be reformed [84]. Physical gelatin gels are not stable because of their thermal reversibility [85]. It has two types based on extraction process. Type B is extracted from alkaline extraction while type A is produced from acid extraction [86]. Both types of gelatins are used in different industries based on their physicochemical properties [87]. Mostly gelatin is obtained from the bones and skin of pigs and fish [88]. Based on high availability and compatibility gelatin is used in industries and different applications [89]. Gelatin which is extracted from animal byproducts has high barrier and mechanical properties. The triple helical structure of gelatin protein

provides its high physical strength [90]. Gelatin is a mixture of a hydrolysate polymer chain with a different molecular weight of α-chain, β-chain, ϒ-chain, and other molecular substances of lower weight with a molar mass of around 180 × 103, 90 × 103, and 300 × 103 g/mol. Proline, Glycine, and hydroxyproline are the most dominant amino acids [91]. Gelatin is used in pharmaceutical, food, industries, and medical based on gelling properties, foaming, film-forming capabilities, and emulsifying properties, biodegradable and biocompatible properties [92]. In a low or intermediate humidity environment, the gelatin film possesses exceptional barrier behavior against gas, oxygen, and aroma permeation [93]. Furthermore, with a denatured and unique amino acid sequence, the phase behavior of semi-dilute and dilute gelatin solutions can be easily adjusted by external stressors such as ionic strength, temperature, and pH [82].

The tensile property of gelatin is low but can be enhanced by using a chemical or enzymatic crosslinker [94] [95] [96]. Gelatin mechanical properties can also be enhanced by adding reinforcing nanofillers. The nanofillers can be CNTs, hydroxyapatite, clay, GO, nanoparticles, nanofibers and calcium carbonate for preparing nanocomposite for different applications, due to their excellent electrical, thermal, and mechanical properties. Directly combining nanofillers with polymer matrix results in unwanted aggregation and defects caused by Vander wall interaction and a high nanofiller ratio. Two methods are used for the dispersion of nanofillers with polymer matrix known as a covalent and non-covalent modification. This composite possesses greater properties than neat matrices. Gelatin is the most popular biopolymers prepared for these nanocomposites [97] [98] [99].

### 2.6.1 Chemical structure of gelatin

Gelatin is a polyampholyte with both cationic and anionic groups, as well as hydrophobic groups, in a roughly 1:1:1 ratio, which distinguishes this polypeptide. In nature, the gelatin molecule is thirteen percent positively charged (arginine and lysine), twelve percent negativelycharged (aspartic acid and glutamic acid), and eleven percent hydrophobic (comprising isoleucine, valine leucine, and methionine. The rest of the chain is formed from proline, glycine, and hydroxyproline. The

representation (Gly-X-Pro) n is responsible for gelatin's triple helical structure, where X represents any amino acid, primarily methionine, arginine, valine and lysine (6%). One-third of the chain is made up of hydroxyproline or proline (33%), and the other third is made up of glycine (33%). The remainder is other residues. Commercially, gelatin is available as both cationic (gelatin type A, isoelectric point (pI) 7–9, prepared by acid hydrolysis of pigskin type I collagen) and anionic (gelatin type B, pI 4.8–5, prepared by alkaline hydrolysis of bovine collagen) protein [100].

### 2.6.2 Gelatin hydrogel formation

Hydrogels are macromolecular compounds that inflate with water and are synthesized by polymerizing monomers. Within the three-dimension network, there is a notable amount of water is present [101]. Due to their outstanding potential natural hydrogel have attracted great attention and is used in wound healing materials, drug delivery, T.E and biosensors [102].

Typical thermoreversible gels are gelatin gel. Above 40$^{o}$C gelatin solution is obtained and its turn to gels when temperature decreased below the gelation temperature. The gel known as physical gel is formed due to hydrogen bonding [103].

### 2.6.3 Gelatin modification

#### *2.6.3.1Enzymatic and Chemical modification*

Physiochemical properties of gel-like thermal stability and mechanical strength can be improved by many researchers by using various crosslinkers. Various chemicals like glyoxal, epoxide, isocyanates, carbodiimides, formaldehyde, glutaraldehyde, and natural compounds like ferulic and tannic acid and enzyme are used to enhance these properties [104]. Glutaraldehyde is a chemical crosslinker and is widely used to immobilize protein by crosslinking between the carbonyl group of aldehyde and amino acid. This reaction takes place in a pH range of neutral to slightly alkaline. The reaction between the carbonyl compound of aldehyde and the amino group the first intermediate known as carbinolamine is formed. Then schiff base is formed by protonation of the OH group and by removing H2O. Intermolecular and intramolecular crosslinking between gelatin is

achieved by doing this [105]. Transglutaminase is a naturally occurring amine-c-glutamyl transferase found in almost all living organisms. It is an enzyme that is used in numerous applications like the pharmaceutical and food. An e- (ϒ -glutamyl) lysine isopeptide bond formation that occurs between a primary amino group (e.g., lysine) and the glutamine side chain is promoted by transglutaminase [96].

### 2.6.4 Properties of Gelatin

It is composed of eighteen different types of complex amino acids, with proline, hydroxyproline and glycine accounting for approximately 57 percent of the whole, while the remaining 43 percent consists of other notable amino acid families such as alanine, aspartic acid, arginine and glutamic acid [106]. It is made up of seventeen percent nitrogen, fifty percent carbon, 25.2 percent oxygen, and 6.8 percent hydrogen, with a mix of single and double unfolded hydrophilic chains. The structure dissolve into colloids when heat is increased [107]. The temperature below 30 to 40 $^{o}$C is known as a gelatinous state [108]. By boiling a long period of gelatin solution the property of gelatin decomposes and cannot be recovered upon cooling. By varying molecular mass distribution viscosity and gel strength vary and can be affected by pH, temperature and electrolyte. For testing gel strength quality of gelatin manufacture has an important criterion [109]. To increase the viscosity that is rises with increase in concentration, it must be kept below 4$^{o}$C. At 37$^{o}$ C, mammalian gelatin reversibly melted into a solution as the triple - helical structure returns to its coiled state. The hydrogen linked junction zones that often keep the physical gelatin network together [110]. Physical gelatin gels, on the other hand, are unstable at physiological temperatures and above due to their thermal reversibility [111]. It will restrict utilization in applications like drug delivery or T.E where gels must remain stable for a set amount of time before dissolving [112]. Therefore, to address this issue and stabilize the gelatin gels, chemical or enzymatic crosslinking is preferred [113]. A recognizable sequence in gelatin is the amino acid. The generation of amino acids is mostly dependent on the hydrolysis of collagen from animal tissues such skin, tendon and bone [114]. Collagen is composed of three polypeptide chains. The inter-chain hydrogen bond is a triple helix that is woven together and structurally maintained. A suitable chemical pre-treatment will disrupt noncovalent connections to provide

enough swelling and collagen solubilization for gelatin extraction. The triple-helix structure frequently unravels and is broken up by the loss of hydrophobic and hydrogen interactions, which is followed by the dissociation of the molecules into smaller pieces [115]. As a result, it is possible to convert collagen to soluble gelatin by dissolving the hydrogen and covalent bonds that keep the triple-helix shape and converting soluble gelatin into collagen [116]. Dispersion stability, low viscosity characteristics, water retention and affinity are only a few of the excellent physical qualities that gelatin possesses overall. Gelatin is a significant food additive because of its coating, hardness, and reversibility properties, according to Ramos et al. Gelatin is also a thickening and foaming agent, which makes it useful as an emulsifier, dispersant, and clarifier [117] [118].

### 2.6.5 Sources of Gelatin

In the food industry hydrocolloids are mostly used as a gelling agent [119] [120]. From plants, most of the hydrocolloids are extracted while gelatin is obtained from animals. Primary sources of gelatin are pigs, fish, and cows. Gelatin and hydrocolloids are confused because some scholars support another group of hydrocolloids, particularly the veggie gelatin plant. Therefore, demineralized cattle bone, bovine skin and pig skin are the only commercially viable sources of collagen [121]. Gelatin is the primary protein found in the skins, smooth connective tissues, and bones of some mammals. It is a hydrolyzed form of collagen. Therefore, the primary sources of animal byproducts used to make gelatin are bovine (derived from cows) and porcine (pig-based) [121].

All hydrocolloids are not plant-based because gelatin is particularly obtained from animals. Gelatin which is extracted from fish skin and scale having proteins 85 to 92%, water, and salt. Gelatin can be found in the bone, connective tissue, scale, skin and intestine of animals via a partial hydrolysis process, according to Jakhar et al, it is of high molecular weight. Lestari et al named the plant gelatin “veggie gelatin”. The gelatin obtained from fish has low melting point and high dissolving rate. Based on raw materials and processing conditions Fish-based gelatin has the same properties [122] [123].

### 2.6.6 Extraction of Gelatin

Beef hides, bones or Porcine skins were considered type A gelatin in the industry, and type B gelatin was commonly extracted occasionally from pig bones and from beef-based raw materials. Caustic lime or sodium carbonate were common alkaline agents and acids used in the industry to extract bacteria and minerals from these raw materials. It shows that type A gelatin is the result of acidic treatment while type B gelatin is result of alkaline treatment. Typical processes use to extract the gelatin particularly porcine and bovine based gelatin is as follows:

1. In degreasing 2% fat can be reduced by after cleaning by soak them in warm water before roasting for thirty minutes at 100$^{o}$C.

2. In the pretreatment soaked them for 5 days in vats of acid or alkali

3. During extraction, the resulting is placed into the extractor, boiled in distilled water, and then sterilized by flash heating for around 140$^{o}$C in 4 sec.

4. In evaporating separate bits of tissue, bone, or skin that are attached can be piped through filters. The filtered liquid was piped into evaporators to separate it from the solid gelatin.

5. In grinding ,gelatin is a grind to powder by pressing them in sheets [124].

According to Gomez-Guillen et al., the acquired pre-treated fish, poultry, or animal skin by-products are in a mixed state between immature liquid gelatin and insoluble native collage at the early stage of gelatin extract ion [125]. To gain the optimal yield of gelatin then this preheated material is further boiled in water above 45$^{o}$C. During pretreatment chemical applied are NaOH and HCl on collagen that breaks the non-covalent bond to disorganize protein that causes solubilization and swelling. The extra heat treatment is now destabilizing the triple helix by rupturing the covalent and hydrogen bonds, which leads to the formation of the helix-to-coil transition and conversion to soluble gelatin. The degree of collagen's conversion to gelatin is correlated with the intensity of pre-treatment and boiling procedures, which are often depending on temperature, pH, and extraction time. [126] [127] [128].

### 2.6.7 Application of Gelatin:

Gelatin is a major element in the food industry and modern cookery because of its gelling characteristics. Gelatin is used in the food processing industry to stabilize food products. and to enhance texture, foaming, and clarity [129]. To retain juices loss of canned product gelatin is used and provide good heat-treatment during cooking. For confectionary products such as ice cream and fruit salad edible gelatin are used [130]. Because of its outstanding nutritional value and film-forming capabilities, gelatin can be employed in edible films and coating materials [131]. As gelling material gelatin can be used in heath products and cosmetic such as bath salt, body lotion, proteins, facial cream and shampoo [132]. Gelatin is used for the cell transplantation carriers, hydrogel, and nanofibers in the medical industry. In different drug products, gelatin is used because of its nano and microparticles [133] [134] [135] [136]. In the medical and pharmaceutical industries, gelatin is used as a matrix for intravenous infusions, injectable drug delivery microspheres, and implants. Bone health and joint can be improved by using the oral consumption of gelatin. In hemostasis, gelatin is very important because at the bleeding site it acts as a stable clot [137]. To stop the blood flow and produce a mechanically stable matrix around the bleeding site, a fibrin clot can develop on expanding gelatin particles. Injectable gelatin microcryogels (GMs), which transport cells into the deep layer of wound tissue, have also been developed by Zeng et al. to improve cell therapy for the treatment of deep wounds. Injecting a deep wound layer with a micro-syringe needle lowers site-specific and minimally invasive side effects [102] [134].

Gelatin can be used to filter out airborne microorganisms. Gelatin filters can be used for catching submicron particles by dissolving in water and sterilization by gamma radiation and wrapping and packaging in a polyethylene bag and this is a very easy and low-cost method for air filters [133]. The reduction of sugar crystal can be controlled by gelatin it also hinders the separation of water and oil in the syrup. In the production of confectionery, a binder is employed as an emulsifier to lessen facilitate cutting, brittleness, and facilitate moulding. This reduces breakage of the different varieties of confectionery and raises the rate of completion. Gelatin also has innovative and appealing applications for forensic research including lifting shoe prints [138]. To eliminate the impressions founded by investigation team in crime

scenes gelatin lifters are used. Gelatin-lifting companies apply a thick layer of gelatin on a flexible woven textile and place it on a product that needs printing. Despite the fact that pressure can imprint objects successfully, it is nevertheless important to avoid applying too much pressure to the lifters because this would distort their shape. In the fabric imprints and fingerprints gelatin lifters are used [138]

Due to colorless, odorless, and tasteless nature gelatin is very useful. It can create a strong, adhesive force and a solid, translucent gel. Due to these properties, it is very adaptable. It is used in cooking as a gelling ingredient, and because of its characteristics, it works well to hold jelly together when it is cold. When wine and beer were brewed it is used as a cleaning agent. To make gelatin taste delicious, it is frequently processed and colored with flavoring, sugar, and preservatives. The health of human may not be always benefited by this. Gelatin is consumed in this fashion because it has evolved into a form that not only looks wonderful but also tastes well and makes the user feel happy. Unflavored gelatin, which is free of preservatives and other sugars, may have a number of health advantages [102] [139].

However, in individuals undergoing heart surgery, gelatin solutions may negatively impact renal function. A recent study by Charles-worth et al. suggests that the perioperative usage of gelatin-based treatments should be reevaluated. Because they think there is growing evidence that any (possible) advantages are outweighed by the risks. They argued that the alleged benefits of gelatins, such as the prevention of hypotension, could be obtained by other methods and that anaphylaxis brought on by their usage was severe, delayed, and possibly more often than epidemiological studies suggested [140]. Regarding the uses of fish-based gelatin, cold-water fish gelatin has limited action as a gelling agent due to its low gelling strength and low hydroxyproline concentration [141]. According to Wasswa et al., there are two main factors that affect the production of fish gelatin: the first is the small amount of intra- and extra-chain non-reducible crosslinks, which will make the collagen in fish skin more susceptible to degrading, and the second is the inherent variation in collagen molecules among the various species of fish skin [142]. Lower proline, amino acids, and hydroxyproline are all present in the gelatin extract from cold water fish. While the fish's functionality

and purity determine the gelatin fish's quality. Fish gelatin also has an osteotropic purpose. Osteoporosis typically affects persons who are at risk for disability and bed rest [143]. As a result, fish gelatin encourages tissue regeneration that can raise bone marrow density and offer patients with osteoporosis additional benefits [144].

Furthermore, warm-water fish gelatin possessed characteristics that were similar to those of pig gelatin. According to Tongnuanchan et al, fish gelatin has outstanding tensile properties, good biocompatibility, excellent film forming properties, non-toxicity, and high quality, making it capable of manufacturing biopolymers that are partially hydrolyzed for the production of biodegradable packaging films. Because of this, working with water can readily impair mechanical strength, which may limit gelatin's ability to be hydrophilic. Wax, fats, and oils, among other hydrophobic substances, are employed to improve their water barrier properties to address this issue [145].

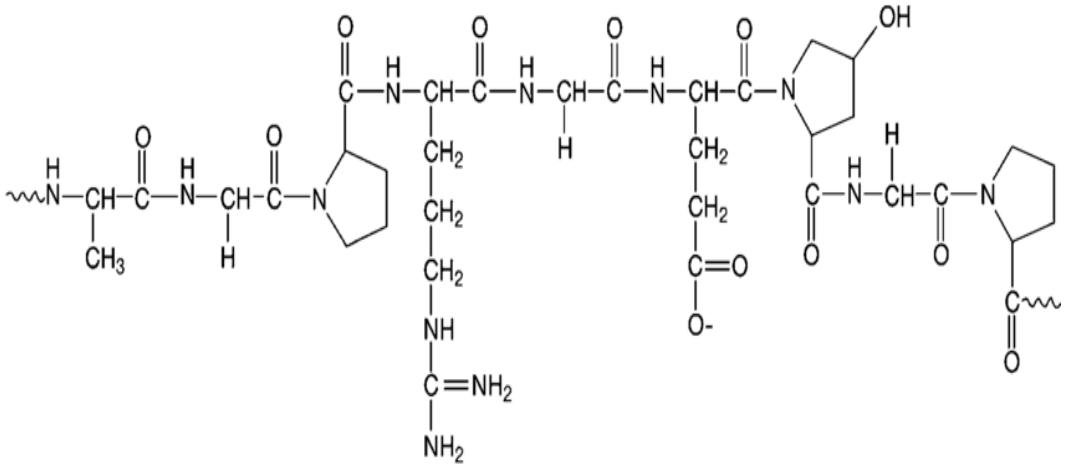


*Figure 1 Structure of gelatin*

## 2.7 Sodium Alginate

A common natural polymer called alginic acid is typically formed from brown seaweeds such as macroalgae, ascophylum, gulfweed and kelp. [146]. Alginic acid is a substance that occurs naturally in the cytoplasm and is crucial for preserving the integrity of the cell wall [147]. According to Myklestad research, alginic acid is primarily present in cells as calcium alginate, but it is also present in little amounts as sodium alginate, magnesium alginate, and potassium alginate. The most common

seaweed extracted from this is sodium alginate. Numerous academics have undertaken extensive long-term research on the usefulness of sodium alginate since it was discovered in kelp in 1883. In the United States, the food industry started implementing large-scale industrial manufacturing in 1944, although the pharmaceutical industry did not start using it until 1929, or roughly 30 years later. The American Food and Drug Administration approved the authorized use of sodium alginate as a food ingredient in 1983 [148]. With the ability to thicken, suspend, emulsify, stabilize, form a gel, form a film, and spin fibers, sodium alginate is a light yellow or white powder that dissolves in H2O to create a highly gelatinous solution. It is used in the food, paper, and cosmetic industries [149]. Alginic acid possesses anti-anaphylaxis effects, immunomodulatory activities, antioxidant activities, and anti-inflammatory properties, according to contemporary pharmacological investigations. Alginic acid obtained from brown algae has also reportedly been shown to have hypocholesterolemic and antihypertensive properties. In literature reveals that alginic acid has been used in a variety of industries, including catalysis, medicine, water treatment, packaging, the food sector, and immobilized cells [150] [151] [152] [153] [154] [155] [156] [157] [158] [159] [160].

### 2.7.1 Structural or chemical properties

The chemical nature of alginic acid is intricate, and the structures of alginic acids obtained from various species vary. In general, the monosaccharide content, position of glycosidic linkage monosaccharide sequence, solubility, glycosidic linkage configuration, and rheological properties of polysaccharides describe their chemical structure [161] [162] [163]. Alginic acid's chemical structure has only been somewhat studied in the past. Methylation analysis One- and two-dimensional nuclear magnetic resonance, assessments of viscosity, and Fourier transform infrared spectroscopy, IR spectroscopy were the principal methods utilized to study alginic acid [164] [165].

The breaking of glycosidic links by derivatization, detection, acid hydrolysis and quantification with Gas Chromatography and High-Performance Liquid Chromatography are typical steps in the investigation of the monosaccharide content. Two structural isomer residues, β-d-mannuronic acid (M) and α-l-guluronic acid (G),

make up sodium alginate. Within the larger polymer of alginate, M and G are joined through 1-4 glycosidic linkages either homogeneously or heterogeneously to produce linear dimers: There are GG and MM homodimers as well as MG/GM heterodimers. As a polymer substance, alginic acid's performance is influenced by the amount of G and M. The molecular structure of a molecule is determined by various G/M unit ratios. The biology and characteristics of alginic acid are determined by various structures and diverse conformations. Alginates with more GG blocks than MM blocks have a higher water solubility. Alginates with more MG/GM blocks are more soluble at low pH levels than those with MM or GG block concentrations [166] [167] [168] [169] [170].

The GG content can influence gelation by interacting with divalent ions like calcium (Ca2+). In general, sodium alginate's G content is recognized as one of its physical and chemical characteristics. Low G content alginate hydrogels are more elastic, while high G indicates the content of G is higher than 70%, the length of the G chain is long, and the stiffness is high. Therefore, altering the product's M:G ratio allows for the creation of gels of various strengths. HPLC can be used to determine the M/G ratio. The study discovered a relationship between the source and season of alginate collection and the ratio of G to M and molar mass of alginate. Varied source materials used to extract alginic acid result in different M/G ratios (mannuronic acid to guluronic acid). The M/G ratio, which measures the ratio of mannuronic acid to guluronic acid in a variety, varies across geographical locations [171] [172] [173].

### 2.7.2 Average molar mass

Its relative molar mass is approximately 32 kDa-200 kDa, and theoretically, the structural unit's molar mass is equal to 198.11. The physicochemical characteristics of alginate are significantly influenced by the molar mass and is represented by the molecular formula [C6H706Na] n. When agitated and injected, high molar mass sodium alginate will create a viscous solution with a high shear force that will seriously harm both raw materials and people. As a result, the choice of a reasonable molar mass allows adjustment of the gel's viscosity and stiffness [174] [175] [176].

### 2.7.3 Characteristics of alginate

Alginate possesses a significant amount of biological activity, however its physiological activity is restricted by its poor water solubility, large molar mass, and extensive molecular chain. Research on the decomposition of polysaccharides can provide the chance for the thorough development and use of their physiological activities, which can help to increase the medicinal potential of polysaccharides. In order to treat sodium alginate, Lee et al. used 60 Co-radiation. As the radiation intensity increased, the degradation rate gradually decreased, the degree of molecular chain breakage increased, the M-M and G-G fragments increased, and the degradation rate gradually decreased. The level of radiation ranged from 10 to 500 kGy. The degradation impact was strongest at 100 kGy of radiation intensity, and it was highly noticeable when sodium alginate's molar mass declined from 300 kDa to 25 kDa. Additionally, due to its easy operation and environmental safety, ultrasonic degradation is regarded as a good way to breakdown alginate. According to Feng et al., when alginate is subjected to ultrasonic treatment, its molar mass steadily lowers as the ultrasonic frequency is raised. The lowest value of alginate molar mass degradation can reach 99 kDa at a frequency of 40 kHz. Alginate was made from Laminaria digitata fronds by Chand et al. and was directly methylated in nitrogen atmosphere using methyl sulphate and aqueous potassium hydroxide. The viscosity of the methylated material was then determined after it had been transformed to methyl ester. Alginate molecules shrank to 3 kDa, an extremely low molar mass value [177] [178] [179].

### 2.7.4 Chemical structure formatting:

A little amount of structural information was released in addition to their monosaccharide components and molar mass. First, it was heated with HNO3, where it underwent hydrolysis and underwent oxidative degradation to reveal its chemical structure. The findings show that the CH3- groups were bonded to either Carbon 2 and Carbon 3 or Carbon 4 and Carbon 5. After being methylated, alginic acid was treated under pressure with methyl-alcoholic hydrogen chloride. The findings indicate that the CH3 groups were located at residues Carbon 2 and Carbon 3 of the mannuronic acid. Thus, all findings suggested that mannuronic acid residues in free C2, C3, and C4 form the chain of alginic acid. The main structural feature of alginic acid is a chain of 1, 4-linked β-D-mannuronic

acid residues, according to hydrolysis experiments with formic acid. Alginic acid was thought to be a polyuronide mostly made up of D-mannuronic acid and L-guluronic acid based on the findings of partial acid hydrolysis. Alginic acid is made up of 1, 4-linked mannuronic acid and 1, 4-linked guluronic acid units, according to the findings of the methylation analysis. Methylation, reduction, and hydrolysis were used to study the structure of alginate, and the results revealed that alginate was linear and lacked any 1, 3- or 1, 2-linkages [180] [181] [182]. Alginic acid was conformed to be a linear copolymer with homopolymeric blocks of 1-4 linked β-L-gulopyranuronic acid (G) and 1-4 linked α-D-mannopyranuronic acid (M) residues. The two uronic acids can be found in heteropolymeric and homopolymeric blocks. X-ray diffraction and polarized infrared were two techniques used to study the chemical structure ofalginic acid's structural components include poly-β-D-mannuronic acid and poly-α-L-guluronic acid, both of which have crystalline structures. In the 1e-4e linked D-mannuronic acid chains that make up poly-β-D-mannuronic acid's structure, monosaccharide units are arranged in the C1 chair conformation. The chain packing and the side (- COOH) group's orientation are different, which affects how the chains are bound together [183].The 1-4 diaxially connected L-guluronic acid residues make up the poly-α-L-guluronic acid chains. Alginate was partially acid hydrolyzed to provide three fractions, and one- and two-dimensional NMR techniques were used to characterize the three fractions. The findings revealed that alternate heteropolymeric block, 2D 1H/1H, made up the majority of fraction 1. A regular homopolyguluronic block structure was present in fraction 3 as well as a tiny number of guluronic acid residues in fraction 2 and it was seen in a 1-4 homopolyguluronic sequence in the NOESY Nuclear Magnetic Resonance spectrum [179] [184].

### 2.7.5 Conformational features

The biological behavior of polysaccharides depends on their chemical composition, molar mass, and chain conformations. There are, however, not many studies that describe the structural characteristics of alginic acid.

The pyranose ring structure or the furanose ring structure must be present in the glycosidic connections with the -configuration 4. The ribbon-like two-fold conformation and

antiparallel arrangement of ribbon-like chains, which are tightly packed and joined into sheets by hydrogen bonds, are the dominant characteristics of polymannuronic acid. The two-fold helical shape is the primary characteristic of polyguluronic acid. The two molecules' conformations are both maintained by intramolecular hydrogen bonding [149] [179] [182]. It is challenging to establish a connection between biological processes and chemical structures and chain conformations. To further understand the complex chain conformations of alginic acid, cutting-edge techniques including nuclear magnetic resonance, SEM, TEM viscosity studies, static and dynamic light scattering, and fluorescence spectroscopy are needed. [185].

### 2.7.6 Physiochemical properties

#### *2.7.6.1 Solubility*

A viscous liquid is formed as sodium alginate is dissolved in water and after reabsorbing water its volume increases up to ten times. By varying the concentration and degree of polymerization viscosity of sodium alginate solution may vary. Sodium alginate solution has an opposite charge because it condenses the hydrophobic suspensions while preserving the negative ion group.

#### *2.7.6.2 Miscibility*

Sodium alginate is compatible with thickeners like guar, tragacanth gum and xanthan, synthetic polymer medicines like sugars, lipids, waxes and carbohydrates some surfactants, and some organic solvents like propylene glycol, ethylene glycol and glycerin etc. It is incompatible with ethanol at concentrations more than 5%, calcium salts, crystal violet, acridine derivatives, heavy metals, phenylmercury vinegar [186]**.**

#### *2.7.6.3 Stability*

Most additive compounds are compatible with sodium alginate (except positively charged molecules). The dry powder state of sodium alginate is more stable than the solution state.

Sodium alginate gradually transforms into an alginic acid gel when the pH level is dropped; when the pH level is increased, the alginic acid dissolve and restores the viscosity to its original conditions [187] [188]**.**

Short period of high alkalinity can be withstood by sodium alginate but longer period of high alkalinity effects upon its viscosity and reduce it [189].

Short term high temperature can be withstood by sodium alginate, but its viscosity can be reduce by long term high temperature [190] [191].

## Gel properties

A decrease in pH will cause alginic acid to gel. . This gel has a low strength, forms a soft gel, and is soluble in alkaline solution. Calcium dioxide (Ca2+) replaces some of the sodium and hydrogen ions in the alginate solution to create a calcium alginate gel when a small amount of it is added to the mixture. The G block is at the core of the three-dimensional network structure that the Ca2+ image structure produces, which is shaped like an "egg-box."

The fact that calcium alginate may form a gel that is heat-irreversible distinguishes it over other colloids. Other than magnesium and mercury, divalent metal ions can quickly react with sodium alginate to generate alginate gels. The gel film made with calcium chloride has the greatest strength.

For the greatest gel performance, a 3 percent sodium alginate solution and a 5% calcium chloride solution are gelled at 60 °C. The M/G value, sodium alginate concentration, combined calcium content, and gelation circumstances should all be distinct from the gel characteristics of the produced gel [192] [193] [194].

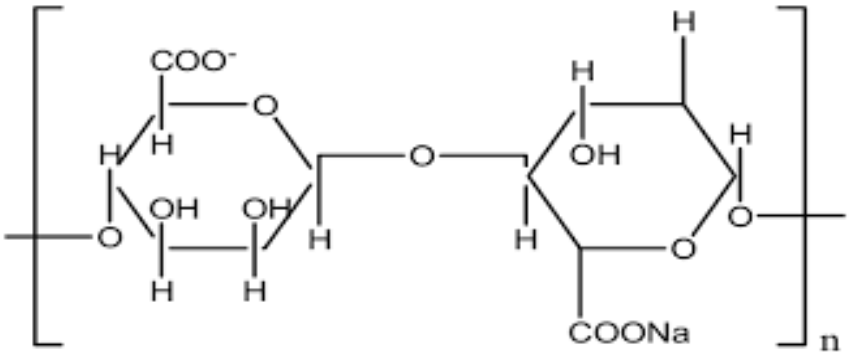



*Figure 2: Structure of sodium alginate*

## 2.8 Bioceramic

As an alternative to metallic implants, bioceramics—materials with synthetic or natural origin—have begun to gain popularity [195]. Ceramics are classified as metal and metalloid oxides, nitrides, sulphides, and carbides [196]. Because they have good and compatible physio-chemical qualities with particular human body components, these are significant in the biomedical sector. Initially, crown treatment in the 18th century employed bioceramics porcelain. Plaster of Paris was then applied to dentistry in the 19th century [197] [198]. Due to improvements in processing technology, ceramic applications in the medical field expanded in the 20th century [199]. Bioceramic materials improve metal-based biomaterials in terms of improved mechanical capabilities, poor degradation, non-corrosiveness, great biocompatibility, and high melting temperature with poor flexibility. Compared to bone, bio-ceramics have a lower elastic modulus, fracture toughness, and hardness [200] [201] [202] [203]. In relation to synthetic bio ceramics like Ca-P based porous materials, zirconia, alumina, titania, and bioactive glasses/glass-ceramics are employed in many different applications including medical sensors, coatings, orthopedics dentistry, calcified tissues, and implants [204] [205] [206] [207].

Additionally, the application of bio ceramics opens up new possibilities for soft tissue regeneration and repair. Bio glasses and glass-ceramics have become popular as a flexible material that can be moulded to fulfill the needs of the user. About 1.5 million bioglasses and glass-ceramics are produced annually at a cost of $10 billion, and demand for them is growing significantly [208]. Bio glasses made of silica have been the subject of substantial research in recent years. Innovative methods for many applications are provided by borophosphate, boronate, borosilicate, and other combinations with various therapeutic element-based compositions. Additionally, this will lead to a greater uptake of bio glasses and glass-ceramics in the orthopedic and dental industries. The use of bio glasses/glass-ceramics as 3D scaffolds, as coatings for implants, and composites with mechanical properties similar to those of natural bones are other advanced techniques [208]. For the right selection of materials, their process parameters, constituents, design, concentration, and balance between diverse attributes is therefore necessary. Dopants in the matrix, for instance, could improve biocompatibility and antibacterial activity in the case of bio glass. However, a toxicity in the body can result from a high dose of doped ions. Biomaterials

made of ceramic have some advantages over alloys and metals, including high elastic modulus, low cost, hardness, and porosity. They do, however, have several drawbacks, such as ductility, low sinterability and machinability. These characteristics limit their applicability as biomaterials. These bio glasses and glass-ceramics with synthetic origins are typically made with expensive minerals. Recently, cost-effective value-added biogenic silica nanoparticles and bio glasses/glass-ceramics have been created using sustainable natural bio-ceramics such as agro wastes/ashes (rice husk ash (RHA), sugarcane left ash (SCLA), and corn cob (CC)) [209] [210]. Similar to this, food wastes like egg shell membrane(ESM), egg shell powder (ESP) and banana peel (BP), which contain polyphenolic compounds, essential amino acids and potassium (K), polyphenolic compounds, can be used to produce hydroxyapatite (HAp), mesoporous bioglasses, an alternative to synthetic bioglasses used for prosthetics and medical treatments, as well as β-tricalcium phosphate (β-TCP), biphasic calcium phosphate [211] [212] [213]. Ashes and synthetic minerals coupled with agricultural waste have demonstrated their ability to replace biological materials in a variety of biomedical applications.

When compared to synthetic bio glasses, bio glasses/glass-ceramics made from agro-food wastes offer greater advantages. To meet the requirements of a biomaterial, the choice of basic ingredients and their quantity are crucial. The shortcomings of ceramic materials were improved and overcame by further process parameter changes and the development of various synthetic technologies.

### 2.8.1 Synthetic bio ceramics

The classification of synthetic ceramic materials into bioinert, bioactive, and bioresorbable subsets reflects the different physiological interactions that body implants and tissues may experience. Synthetic bioceramics are utilised to create artificial plants, heart valves, stents, tooth fillings, and medication delivery systems. The development of process technology makes it possible to adjust the physical, structural, and mechanical characteristics to the requirements of the body parts and applications. The synthetic ceramic biomaterials were described in the following sections in light of their use in healthcare [214].

#### 2.8.1.1 Bioinert ceramics

Ceramics that are bioinert do not react biologically with the tissues around them. When exposed to a physiological environment, it can resist corrosion. Alumina and partly stabilized zirconia are examples of oxide bioinert ceramics, while carbon and nitride-based ceramics are non-oxide bioinert ceramics [214].

##### *2.8.1.1.1Alumina*

Dental and orthopedic implants typically employ alumina. It is biocompatible with the human body and has good mechanical strength, a low coefficient of friction, and biocompatibility. Materials with small grains and low porosity shown strong mechanical strength. Some tensile properties of materials are dependent on grain size and porosity [205]. Zhao et al. created alumina with a grain size of 1μm and a porosity of 0.7 percent, which gave it a translucent appearance. For the particular implants, porosity encourages osteointegration and bone development [215] [216] [217]. By improving the solid-state diffusion process, the addition of calcia or magnesia (MgO) allowed the alumina to be sintered at a temperature lower than 1600 C [217]. Alumina is also utilized to create nanocomposites, which are then studied for their cytocompatibility, biomechanical, and m-RNA gene expression behavior using a computerized histology index. These materials include graphene platelets, alumina/Ti and hydroxyapatite (HAp) [218] [219] [220]. Implants made of alumina are unable to adhere to both soft and hard tissues. SiAlON-Al2O3 ceramics produced using the direct nitridation process offer the necessary porosity, compressive strength, and biocompatibility to address this problem [221].

#### *2.8.1.2 Zirconia*

Zirconia is an allotropic oxide that, depending on the temperature, forms in 3 different crystal structures: cubic(2680$^{o}$C), monoclinic (1170 °C) and tetragonal (2370 °C). It has been noticed that other oxides, including CaO, MgO, CeO2, and yttria, are utilized as stabilizers for zirconia (Y2O3). According to several investigations, the combination of Y2O3 stabilized titania nanotubes and zirconia demonstrated a novel method for examining friction and wear rate for orthopedic applications [222] [223] [224] [225]**.**

### 2.8.2 Bioactive ceramics

Bioinert and bio-resorbable ceramic materials' characteristics can be shown in bioactive ceramics. The term "osteoconductivity" or "bioactivity" refers to these materials' capacity to connect with bone. Due to their osteoconductive qualities, these bioactive ceramics are also employed as coating materials to improve the corrosion resistant and mechanical qualities of bone transplant implants. On their surfaces, they can also serve as a scaffold to promote bone development. CaP, HAp bio glasses and bioactive glass-ceramics are the most popular bioactive ceramics. The next section goes over and summarizes these bioactive substances [214].

#### *2.8.2.1 Bio glasses*

Hench et al. [226] made the discovery of bio glass (45S5) (BG) in the early 1970s for use in replacing bone. Following its discovery, many bio glasses have been reported for use in a variety of applications, including the treatment, heating, implants, and fillers [207]**.** The original BG composition, which is based on silica (Si) as a glass forming with bone-bonding properties after post-implantations in vivo, is 45SiO2-24.5CaO-24.5Na2O-6P2O5. The bonding processes of BG to biological tissue encompass a series of eleven chemical stages [227]**.** The (hydroxycarbonate apatite) HCA layer is formed on the surface of glasses in a process that Hench defined as consisting of 1–5 steps. On the basis of these methods, BGs are discovered to be "bioactive". When BG is dissolved in a physiological environment, two things happen:

1. On the surface an apatite layer forms, and
2. Physiologically active ions are released into simulated bodily fluids or plasma during in-vitro and in-vivo testing, respectively [226] [228].

### 2.8.3 Bioresorbable ceramics

Various medical specialties use bioresorbable ceramics as a substitute for polymeric and metallic biomaterials. Due to the release of acidic byproducts into the human body, biodegradable metals and polymeric materials cause localized irritation [229]. A substance that dissolves after being inserted into a human body and is progressively replaced by developing tissues is referred to as bioresorbable (bones). The most typical types of bioresorbable ceramics include porous HAp calcium phosphate, calcium sulphate

and their salts. These ceramics are frequently used to repair broken bones without inflaming them [230]. Materials that are both bioactive and biodegradable are frequently researched together, such as CaP and HAp, due to their tight interaction. In contrast to the first generation, which is bioinert, and the third generation, which corresponds to scaffolds used in BTE, both are often regarded as second-generation bio ceramics [231].

#### *2.8.3.1 Calcium sulphate*

Calcium sulphate is a crystalline, inorganic osteoconductive material that exists naturally. It comes in a variety of hydrate forms, including gypsum, anhydrite, and hermihydrate state (CaSO4-.0.5H2O) (CaSO4 .2H2O). Calcium sulphate is used alone, in combination with other chemicals, or as an injectable suspension in the form of pellets. It has a compressive strength that is comparable to cancellous bone (99.15 MPa). When hydrolyzed, it is weak and rapidly loses strength. Due to their injectability and rapid degradability, they have been used as bone transplants and as antibiotic carriers to prevent infection. [232].

#### *2.8.3.2 Calcium phosphate*

For longer periods of time, several CaPs are also utilized to generate artificial bone, including ACp , TCP, FAp, DCP, and HAp etc. When compared to calcium sulphate bone grafts, the CaP-based bone grafts decay more slowly [232]. Depending on their Ca/P ratio, these various classes of CaP materials have varying solubilities and bioactivities. The biodegradation of a substance is influenced by several variables, including its physical characteristics (such as volume, density, surface are porosity) crystal structure, chemical composition and pH stability. For instance, materials with a body center structure (BCC) disintegrate more quickly than materials with a face canter structure (FCC). BCC has an open crystal structure and is less stable than Face Centre structure. Ceramics grain boundaries allow for the release of micro- or nanoparticles when the material is reabsorbed, which can be useful in applications such as BTE and gene therapy [205]. TCP, HAp, and biphasic calcium phosphate are Calcium phosphate ceramics that are frequently utilized for BTE. Tri Calcium Phosphate has two phases, for example, β-Tri Calcium Phosphate and α-Tri Calcium Phosphate. Numerous studies demonstrate that α-Tri Calcium Phosphate dissolves more quickly than β-Tri Calcium Phosphate, however β-Tri

Calcium Phosphate is mostly employed in medicine because of its osteoconductive and osteoinductive properties [204] [233]. Additionally, in-vivo research shows that α-Tri Calcium Phosphate is more resorbable than β-Tri Calcium Phosphate during bone formation for T.E [234] [235]. TCP is more bioresorbable than HAp in both phases. Since sintered HAp is more stable, it can be implanted and stay in the body for a very long time. Due to its chemical resemblance to the inorganic components of bone tissue, HAp is frequently utilized in place of the many crystalline forms of CaP. The mineral phase makes up 69 weight percent of bone, followed by the organic matrix and water (9 percent). Although it has a low bioresorbability, it aids in the formation of apatite crystal nucleating sites in the culture medium [236].

Depending on how it is made, HAp may be natural or artificial. A variety of manufacturing processes can also be used to create HAp powders, each of which results in a different range of particle sizes and shapes [236] [237]. These include sol-gel methods, which produce sintered HAp nanocrystals that range in size from 50 to 70 nm, and hydrothermal processes, which produce HAp whiskers that are 10 μm wide and 150 μm long. HAp ceramics cannot be employed for load-bearing applications in the human body because they have a lower mechanical strength than inert ceramics. Additionally, HAp's phase composition, density, crystal size, sinterability and porosity affect its mechanical properties. Vickers hardness of dense HAp ranges from 3 to 7 GPa, while Weibull's modulus ranges from 5 to 18, both of which are indicative of brittle materials [238]. To improve the mechanical properties of HAp, several elements (Si, Mg, Sr, and Zn) were substituted [239] [240] [241]. Similar to this, other research demonstrated that Carbon Nano Tubes were also substituted in HAp composites, offering the chance to accelerate apatite mineralization, replace collagen fibers as structural stiffeners for bone scaffold and to improve fracture toughness [242] [243] [244] [245]. HAp's biocompatibility is well recognized, yet it remains difficult to employ HAp as a coated metallic implant over an extended period of time. This is a result of thermal stresses created at the ceramic-metallic interface during coating preparations (caused by a difference in the coefficient of thermal expansion) [237]. Although HAp has significant biological activity, it degrades slowly in living things. Poor mechanical qualities and a quick rate of deterioration characterize β-TCP. Due to its progressive breakdown in the medium, the combination of β-TCP and

hydroxyapatite phase, known as Biphasic Calcium Phosphate ceramic, is used as a drug delivery system, regenerative material and repair of complicated bone lesions [246].

BCP, which has a high reactivity, dynamically evolved from a rhombohedral to a hexagonal form [246]. Similarly, BCP produced using the coprecipitation approach displayed increased strength, bioactivity and density for compositions with a greater 70:30 TCP:HAp proportion [247].

Pure TCP and a mixture of 20% HAp and 80% TCP, on the other hand, exhibited little surface reaction. Alginate is added to Ca deficient Hap (CDHA) with BCP to improve its biological performance. Studies conducted in vitro and in vivo with intramuscular implantation demonstrated that Calcium Deficient Hydroxy Apatite with a high level of Biphasic Calcium Phosphate (20% Hydroxy apatite: 80% Tricalcium phosphate) demonstrated superior osteoinductivity and bioactivity. In conclusion, these results show that altering the β-TCP/HAp ratios of BCP ceramics can influence their bioactivity and resorbability [214].

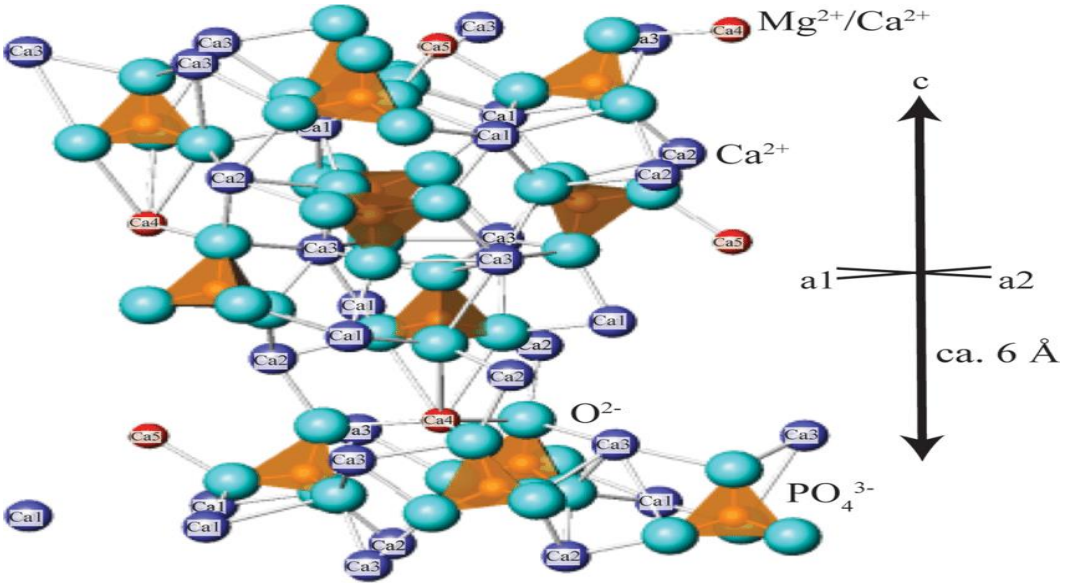



*Figure 3 Structure of TCP*

# Chapter: 3

# Materials and Method

## 3.1: Materials

- Calcium Nitrate
- Ammonium Hydrogen Phosphate
- Sodium Hydroxide
- Gelatin
- Sodium alginate
- DI water

Calcium nitrate CAS [13477-34-4] made in European Union and Ammonium Hydrogen Phosphate CAS [7783-28-0] made in Spain were bought from Scharlau. Sodium Hydroxide CAS [1310-73-2] made in Germany is taken from Merck. Sodium Alginate CAS No:9005-38-3 made in Korea is bought from KOSDAQ. Gelatin CAS No 9000-554-6 is taken from ITW companies.

## 3.2: Methodology

### 3.2.1 Preparation of β- TCP

For the preparation of β- TCP, aqueous solutions of Calcium Nitrate (0.226 M), Ammonium Hydrogen Phosphate (0.15 M), and Sodium Hydroxide (1M) has been taken in 50 mL of deionized water each. Calcium Nitrate was added in the solution of Ammonium Hydrogen Phosphate drop by drop and stirred for 10 minutes with the help of magnetic stirrer. pH value of this solution was maintained at 10 by adding the Sodium Hydroxide (NaOH). Afterwards, solution was magnetically stirred for 4 hours and kept for aging at room temperature (RT) for 24 hours. White precipitates were obtained after

aging, washed with DI water and dried overnight in drying oven at 50°C. After drying sample was grinded in pestle and mortar to obtain powdered sample and annealing was done at 500°C for 6 hours in muffle furnace.

### 3.2.2 Fabrication of sodium alginate and gelatin composite:

Sodium alginate blended gelatin membrane was prepared by using solution casting method. Composition of prepared membrane was adjusted as 90 w/v % gelatin and 10 w/v % sodium alginate in 20 mL deionized water. Gelatin solution was stirred for 2 hours at 40°C while sodium alginate solution was stirred at room temperature for 2 hours. After stirring for about 2 hours, both solutions were mixed, and reaction mixture was homogeneously stirred for additional 2hours. Resulting mixture was poured into sterilized polymeric petri dish and air dried.

### 3.2.3Fabrication of composite with filler:

Mix 1.3gram gelatin in 15ml water at 40°C and stir for 2 hours. Mix sodium alginate in 15ml water and stir for 2 hours. Dissolve different concentration of filler by varying from 0.02 to 0.1g in 10ml water and sonicate it for 2 hours. After 2 hours mix both solution and filler and further stir for 2 hours. Then cast the final solution in a petri dish.

## 3.3 Characterization

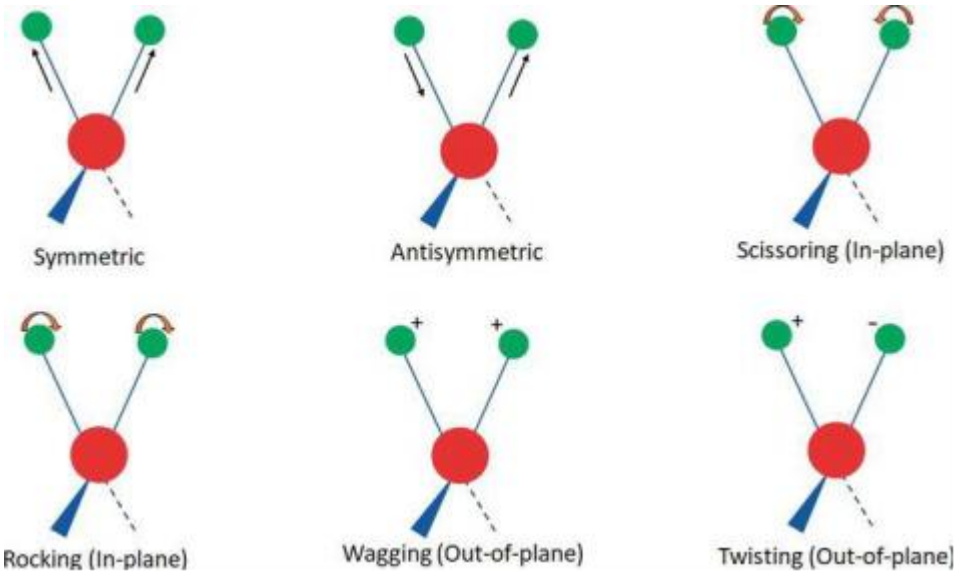
### 3.3.1 FTIR spectrophotometer

To identify the material in the sample, FTIR spectrophotometric analysis was performed. Peaks in the sample's InfraRed spectrum represent the excitation of vibration mode of the molecules in the sample and are thus associated with the various functional groups and chemical bonds present in the molecules. Thus, a compound's IR spectrum is one of its most distinguishing physical properties and can be thought of it as "fingerprint". Because the amount of IR energy absorbed by a molecule is related to its concentration, IR is utilized in quantitative analysis. [248] . FTIR employs low energy IR radiation to cause vibrational movements in chemical bonds and functional groups attached to a compound [2]. The change in dipole movement is necessary for IR spectrum and only those give IR band which have this change. Which molecules like CO2, N2, F2 they do not have dipole

movement and resultant no IR band will be formed [248]. There are two types of molecular vibration produced by IR interaction. The structural geometry, position and orientation of molecules in 3-D govern the changes in vibrational types. Bond stretching vibration will be generated for a linear geometry. There are two types of stretching symmetrical stretching and asymmetrical stretching. The mode of vibration for any complex structure is divided into different bending movements which are as follows [2]

- Scissoring
- Rocking
- Wagging
- Twisting

Modes of vibration in FTIR [248]




The electromagnetic radiation's infrared region is divided into three regions:

- Near InfraRed
- Mid InfraRed
- Far InfraRed

The most used range is mid IR range which extend from 4000cm-1 to 400cm-1. This range has sufficient energy to produce stretches and bends in a functional group for its identification and detection [248].

Any form of sample like solids, liquids, and gases can be identified through FTIR. The phase of matter can decide the preparation of the sample. For characterization of our composite the pellets are prepared. Small pieces of sample are cut down and put into the KBr powder and mixed it. Then this material is processed through the pressure and form pellets. Then these pellets are allowed to pass the IR radiation in an equipment. The IR light produce vibration in a functional group, and these are detected through detector and amplify through amplifier. The signal is recoded and display as a graph in the form of peaks [2].

*Figure 4 FTIR equipment*

### 3.3.2 X-ray diffraction analysis:

X-ray diffraction is used to physically characterize the material. It is a methodology used to find the crystallographic structure of material. It is used to analyze the structure.

Furthermore, XRD analysis can reveal information about the crystal lattice such as crystal system, plane angles and distances, crystal size and structure defects. These are the different methods used for determination of crystal structure.

- Rotating crystal structure
- Laue method
- Powder X-ray Diffraction

Powder XRD is most sophisticated and widely used technique for studying and characterizing material. Cathode ray, sample holder and detector are three main parts of instrument. The tube filament is heated to produce X-ray which results in electron production in the cathode ray tube. The electrons are accelerated in the direction of target material such as copper or molybdenum. Electrons with enough energy will be able to penetrate the target and thus excite the inner electron. The excitation and de-excitation of these electrons generated the X-rays [2]. These characteristics X-rays are bombarded on the sample and X-ray beams obey the Braggs Law and constructive interference occurs and detected by detector in the form of peaks. The Braggs Law is

$$\mathbf{n\lambda = 2d \sin\theta}$$

n= order of interference

θ= angle of incidence of X-ray beam

d=interlayer distance between plans of crystal

λ=wavelength of incident x-ray beam

*Figure 5 XRD equipment*

### 3.3.3 Scanning Electron Microscopy

SEM is a methodology used for producing stunning images of micro and nano worlds. It employs electrical and optical systems to provide detail information about crystal structure, morphology and surface. The SEM scans the material surface with high energy electron beam, and resultant electron radiations by a materials interaction with electron beam are collected and detected to provide an image of material. SEM is used for analysis. In our study, SEM model JEOL JSM-64900 was used to analyze the hydrogel samples and various images at different magnifications were taken from the surface and the cross-section of the samples [2].

*Figure 6 SEM equipment*

### 3.3.4. Tensile Stress Testing (TST)

To find out the mechanical strength of hydrogel sample Linkam Tensile Stress Testing stage (TST350) was used. Young Modulus (YM), Ultimate Tensile Strength, Elongation percentage is calculated by using tabletop apparatus in which samples are fixed between gauges. For our experiment the dimensions 26mm X 7mm was fixed in the gauges of apparatus. The speed was 80mm/min and force applied on it was 20N. Stress was applied according to scan rate until the sample broke and note the readings [2].

*Figure 7 TST equipment*

# Chapter: 4

# Results and Discussion

### 4.1.1 Characterization techniques for β-Tri Calcium Phosphate and composite

We prepared β-TCP and its composite. To understand that is it form or not and the nature of the β-TCP the sample pass through different characterization techniques. Following characterization techniques were applied

- FTIR
- SEM
- XRD
- Mechanical properties

The methodology of each technique is discussed in chapter 3. In this chapter result and interpretation will be discussed.

### 4.1.2 FTIR of β-TCP

The FTIR spectra of β-TCP revealing the absorption at 400-4000$cm^{-1}$ is shown in fig8. The IR characteristics peaks of phosphate groups appeared between 900-1160 $cm^{-1}$. The peak at 938$cm^{-1}$shows the presence of pure β-TCP. The bands between 900-1200 represents the stretching mode of $PO4_3^{-1}$. The peak at 722$cm^{-1}$ attributed to the presence of $P2O7\ 4^{-1}$ group [249]. The peaks between 3500-4000$cm^{-1}$ related to vibrational characteristics of OH bond shows the presence of water. The band at 1034 and 1078 $cm^{-1}$ corresponding to the anti-symmetric stretching of $PO4^{3-}$. 938$cm^{-1}$ shows the symmetrical stretching of $PO4^{3-}$. The band at 571$cm^{-1}$ corresponding to anti-symmetric stretching. The peaks of absorbed water are present at 1638 and 3462$cm^{-1}$. 1385$cm^{-1}$ band corresponded to the absorption peak of CO3-2, which may be due to the absorption of CO2 in the powder of β-TCP [250].

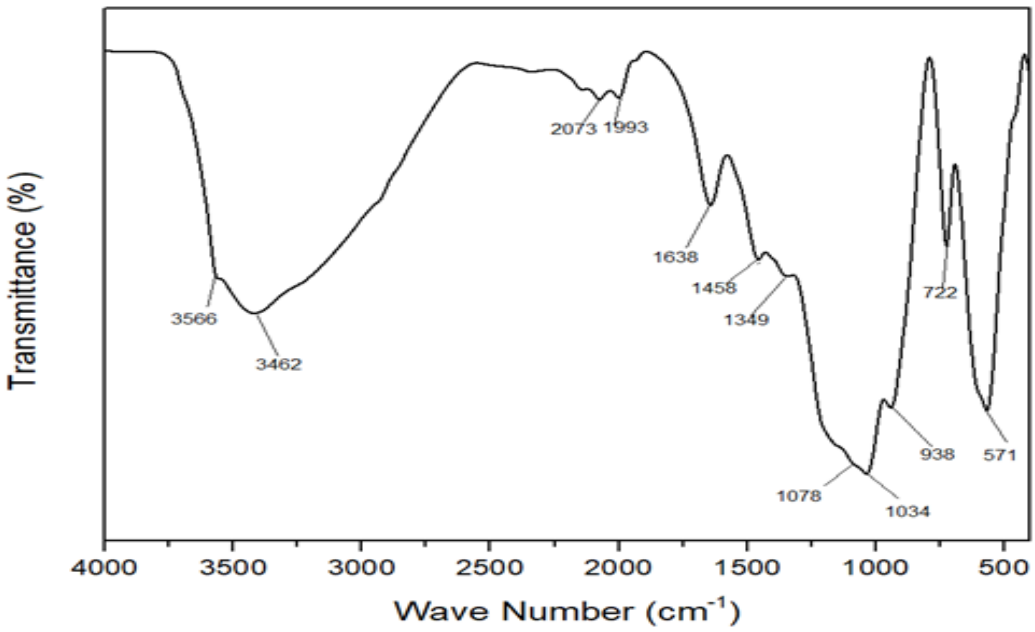



*Figure 8 FTIR of beta-TCP*

**FTIR of composite with and without crosslinking**

The FTIR spectra of gelatin and sodium alginate with and without crosslinking with β-TCP is shown in figure 9. In the blend there is only presence of gelatin and sodium alginate. Then different concentration of β- TCP is added. When we add the β-TCP the peak shift towards red shift [251]. The presence of pure gelatin is shown by the peaks from 1260-1630$cm^{-1}$ [251] [252]. The peaks from 3400-3600$cm^{-1}$ are the O-H stretching peaks [252] and also shows the COO bond in sodium alginate. The N-H stretching in gelatin is attributed to 3270-3370$cm^{-1}$. The stretching of CH bond in amide is present at 2947$cm^{-1}$. The peaks aa 1090-1120$cm^{-1}$ and 560-600$cm^{-1}$ represents the pure β-TCP because these shows the symmetrical and asymmetrical stretching of $PO4^{-2}$. The presence of $PO4^{-2}$ is characteristic of β-TCP. The peak at 810$cm^{-1}$ is the peak of sodium alginate. The peaks between 3400-3600$cm^{-1}$ are the peak of alginate due to COO group [253].

A lot of studies have been done to characterize the compatibility between sodium alginate, β-TCP and gelatin. Dong et al. studied the interaction and good compatibility between the sodium alginate and gelatin. By using FTIR analysis they described the absorption band

related with stretching vibration of N-H group bonded with O-H group shifted toward lower wavelength. They also suggest that hydrogen bonding was increasing [254].

Das et al. suggested the interaction mechanism between the attachment of TCP with sodium alginate. In the presence of $CaCl_2$ the $COO^-$ group of alginate crosslink with $Ca^{2+}$ion by electrostatic interaction and form alginate TCP gel. Alginate connects with other alginate and form egg box pattern. The $Ca^{2+}$ connects alginate chain by ionic interaction and make layers around these egg box structures [255]. The FTIR shows the interaction between gelatin, sodium alginate and TCP. It was observed that hydrogen bonding is responsible for binding these components. The peak which shifts towards lower wavelength indicates physical interaction and good compatibility between components of composites [256].

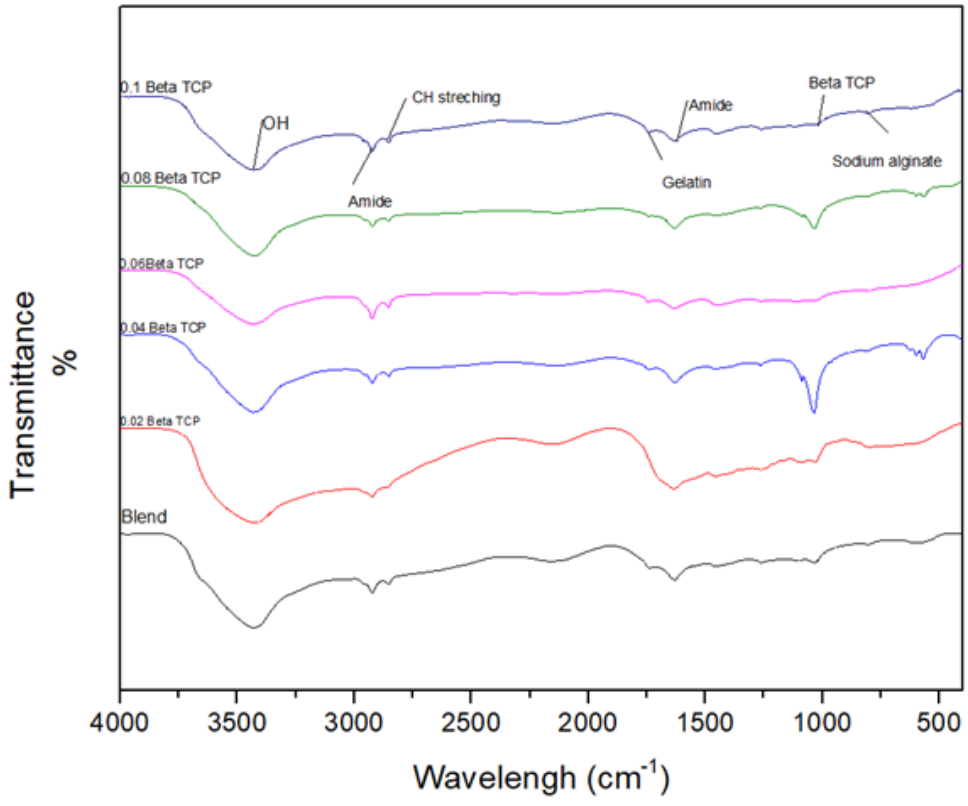



*Figure 9 FTIR of composite*

## Scanning Electron Microscopy

### SEM of β-Tri Calcium Phosphate

Scanning Electron Microscopy images of β-Tri Calcium Phosphate prepared material is present in figure 10. It shows the pore size and pore distribution of β-TCP. It is synthesized by annealing at 500$^{o}$C. As the rate of annealing is increased the particle size will increase [257] this is due to the grain growth which is happening at highest temperature [69]. At both magnifications surface of β-TCP shows roughness and granules. The micro and macropores is due to the pore network which increase the solubility of product [257]. The diameter of particle range is between 20-50nm [258]. The Ca/P ratio of β-TCP is 1.5 [259]. SEM image clearly shows that β-TCP is irregularly shaped small particles and show the accumulation. It is shown that β-TCP is a narrow and homogeneous size distribution despite of ground in mortar [260]. The SEM image show that powder is spherical in shape [261].

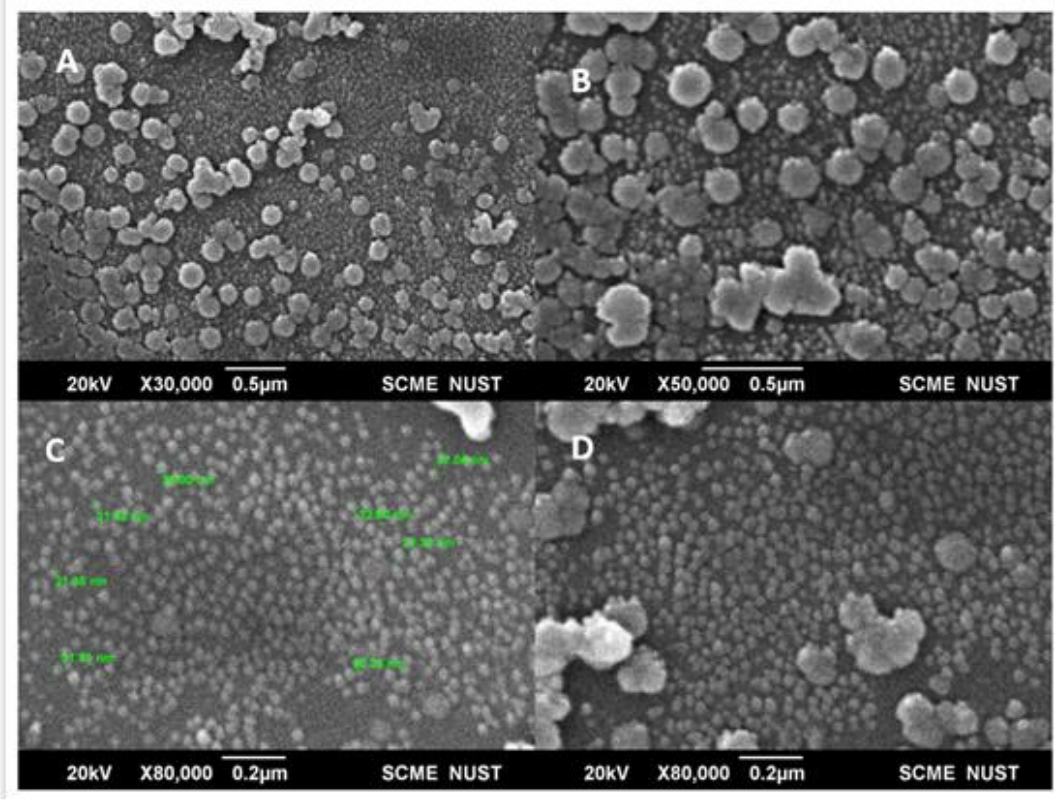



*Figure 10 SEM of beta-TCP*

**Scanning Electron Microscopy (SEM) of composite**

SEM of composite is shown in figure 11 with and without filler. The cross-sectional area and surface of the composite material were determined by using SEM. The image of composite material shows that it is a dense, tight, tough, and wavy surface which indicates the toughness and crosslinking [262]. This toughness increases surface area and the self-healing ability of composite. The surface is rough due to the incorporation of β-TCP. The rough nanofiber surface created by apatite particles has been shown to promote proliferation, osteogenic differentiation and cell adhesion of bone-forming cells [261]. After observation, it can be seen that there is least noticeable porosity in the composite which is due to the addition of β-TCP, that increases crosslinking [263]. By adding β-TCP in composite the pore size can be increase but they lack uniform structure and the refinement of porous structure [264].

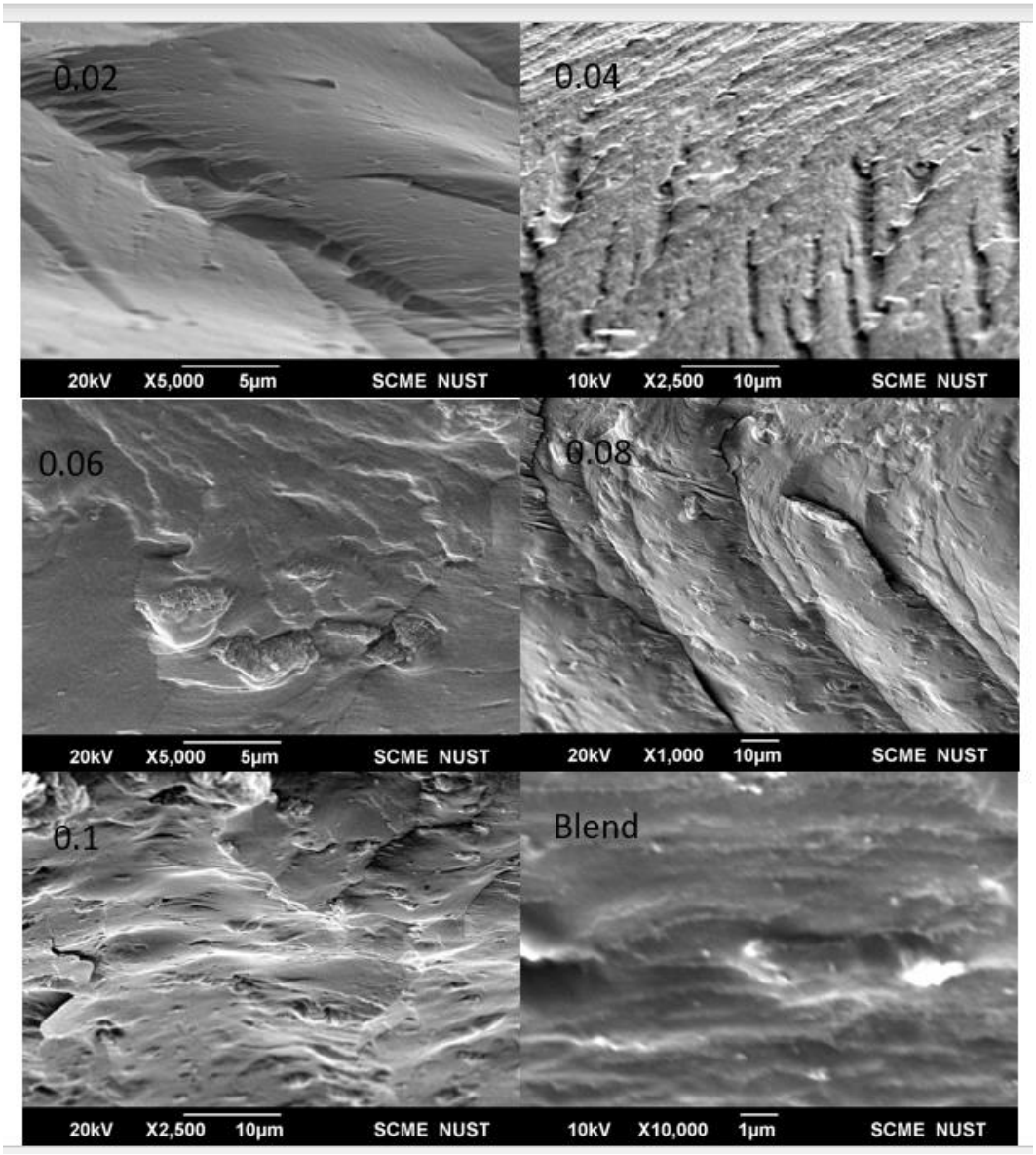



*Figure 11 SEM of composite at different concentration*

**XRD of β-TCP**

X-Ray Diffraction is performed to identify the phases present in the sample according to a Ca/P ratio of 1.5 then the figure is compared with the standard data bank of JCPDS [265]. XRD of β-TCP is shown in the figure. The calcium phosphate obtained is confirmed from JCPDS:09-0169 which is confirmed by a peak at 31.03°. The other remaining peaks

also indicate the formation of β-TCP [266]. According to hkl value the characteristics peak of β-TCP are (0210) and (1010). The peaks are high, intense and narrow. These peaks indicate that β-TCP crystallizes well. There is no impurity in the sample of β-TCP [267].

**XRD of composite**

To find out the crystallinity of β-TCP and its presence XRD analysis was performed. The XRD of composite shows that the gelatin and sodium alginate have no characteristic peak because they are amorphous in nature [256]. So XRD represents the presence of β-TCP in the composite. The peak from 29 to 31 indicate the presence of β-TCP. The values match with the JCPDS card no 00-043-0224 and 09-0169. The characteristics hkl values of β-TCP are 0210 and 1010. These values indicate that β-TCP is crystalline in nature. The values also indicate that β-TCP is fully merged in the composite. The β-TCP shows that it is ceramic in nature regardless of polymeric in nature. It also demonstrates that ceramic is fully merged in the composite [261]. The peak at $2\theta=20$ shows the amorphous nature of gelatin [268]. When gelatin is mixed with the sodium alginate, the peaks were shifted towards $2\theta=31.8$ and $2\theta=43.9$, this is due to the presence of $CaCl_2$ that is formed during the encapsulation process. The loss of the characteristic reflections of ALG and gelatin demonstrated that the structure is weakened by the electrostatic contact between the carboxyl group of ALG and the amino group of gelatins [269].

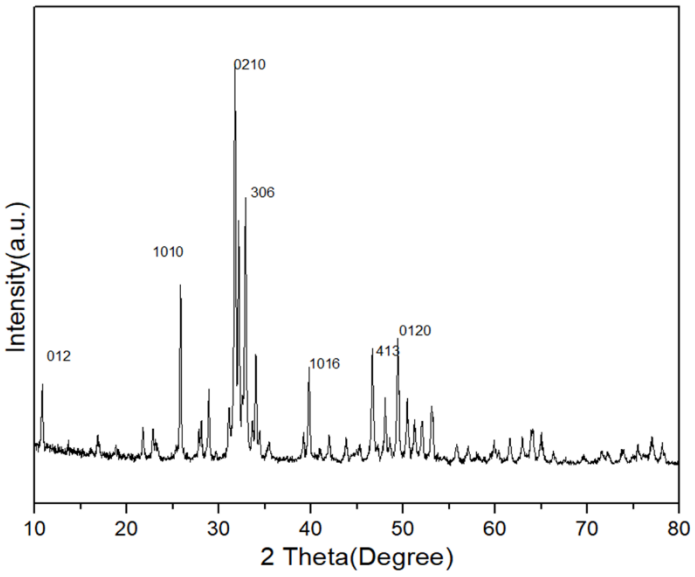



*Figure 12 XRD of beta-TCP*

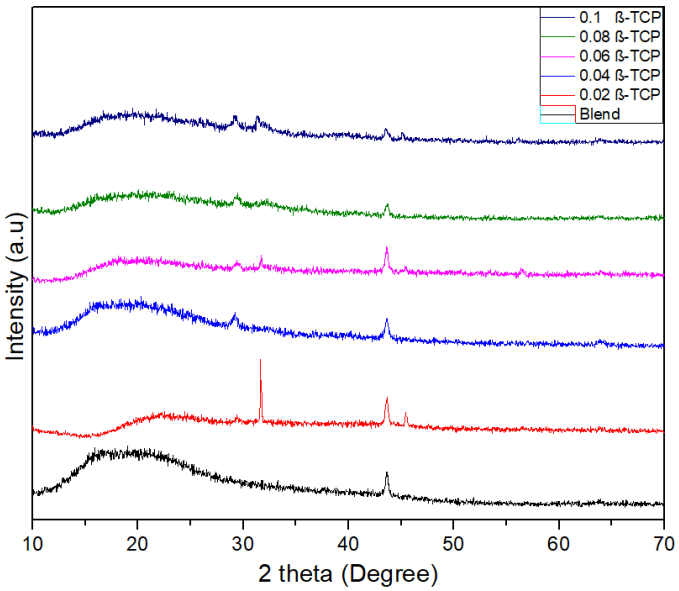



*Figure 13 XRD of composite*

**Contact Angle**

Contact angle is used to measure the hydrophobicity and hydrophilicity of the composite. The materials which have the angle less than $90^o$ are called hydrophilic while the materials having angle more than $90^o$ are called hydrophobic [270]. Another crucial physicochemical characteristic of a scaffold that affects its bioactivity and potential for regeneration is its hydrophilic nature [271]. The hydrophilic qualities of the scaffolds are crucial for cell attachment and nutrient diffusion, a phenomenon that is essential for preserving cell viability, according to the descriptions of various earlier research [272]. Wei et al discovered that as the water contact angle of the material surface falls, osteoblast cell adhesion rises [271]. Generally, surface is hydrophilic when angle is between 0 to $30^o$ and mildly hydrophilic when angle is between 30 to $90^o$. Surface more than $90^o$ are hydrophobic in nature. The results obtained in table 4.1 shows the moderately hydrophilic character since its mean angle is $70.5^o$ [273]. The hydrophilic character is due to carboxyl and hydroxyl groups present in alginate. The other reason of hydrophilicity is due to hydrogen bonding between the glycerol and gelatin. B- TCP also influence upon the hydrophilicity of the contact angle. The composite having higher amount of TCP is highly hydrophobic [274].

*Table 1Contact angle of composite at different composition*

| **Sample** | **CA** | **Hydrophilicity/Hydrophobicity** |
|---|---|---|
| Blend | $72.7^o$ | Moderately hydrophilic |
| 0.02 | $69.9^o$ | Moderately hydrophilic |
| 0.04 | $68.8^o$ | Moderately hydrophilic |
| 0.06 | $86.9^o$ | Mildly hydrophilic |
| 0.08 | $76.9^o$ | Mildly hydrophilic |
| 0.1 | 48.1 | Hydrophilic |

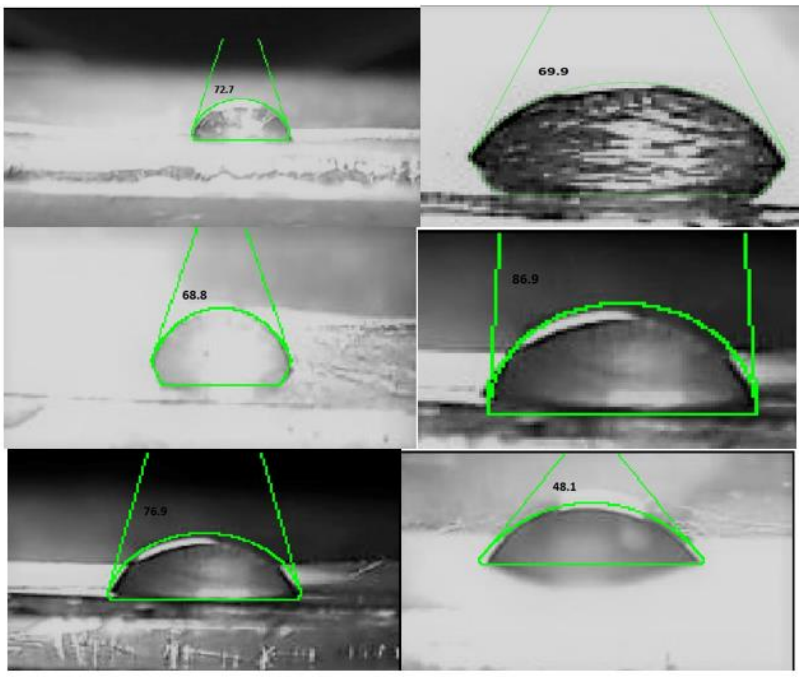



*Figure 14 Contact Angle of composite at different concentration*

**Mechanical of composite**

A key criterion for a barrier substrate that would be helpful during bone development is that it has strong mechanical properties. The corresponding stress-strain curves of the substrates were used to study their tensile mechanical characteristics. Figure 1 shows the nanocomposite samples' Young's modulus and compressive strength data. The UTS, elastic modulus and elongation of substrates were significantly improved as the amount of β-Tricalcium Phosphate rose to 0.04percent. Since earlier studies have demonstrated that an increase in β-TCP can have a significant favorable effect on the mechanical properties, the improvement in mechanical characteristics can be attributed to this. This effect results from the cross-linking of polymer chains, which reduces the size of all the pores. Pore size can increase up to a limit otherwise it decreases the mechanical properties of composite. From micro to nano level the surface to volume ratio increases which has enormous effect upon the mechanical properties. The addition of filler has also affected upon the mechanical properties. Added filler amount from 0.02 to 0.1 g. From 0.02-0.04

the mechanical properties increase. This increase is due to surface to volume ratio and better dispersion of nanoparticles. But when we increase amount from 0.06-0.1 the composite strength starts decreasing this is due to agglomeration of nano particles. This agglomeration decreases the mechanical strength and form weak structure and can be easily break when stress is applied. This leads to microcracks and decrease in mechanical properties. Thus, the best mechanical properties are due to the lower amount of filler as compared to higher amount [275]. β-TCP has positive effect upon the mechanical properties. It reduces the pore size by filling voids and defects. It decreases the stress intensity at microcracks and resists the microcracks. This increases the compression strength due to the interaction of nanofiller with composite matrix. The effect of porosity is indirectly related to mechanical performance. As the porous structure reduce, the mechanical strength increase [276] [277] [278].

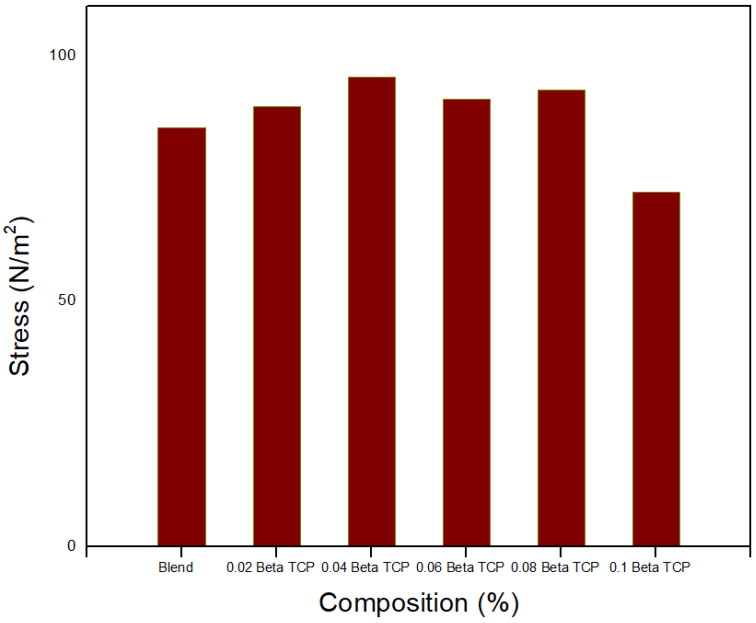



*Figure 15 Mechanical of composite*

# Conclusion

β-tricalcium phosphate was synthesized successfully at pH 10 and was used as a filler to enhance the mechanical properties of composite. B- TCP was characterized through XRD, FTIR, and SEM showing its crystalline structure, composition and spherical structure respectively. Then the composite was prepared by using sodium alginate and gelatin. Further the composite with and without filler was characterized for bone tissue regeneration. The tensile strength of these composites was tested by tensile stress testing. The best results were obtained for composite containing 0.04% β-TCP. And it was observed that the tensile strength of the composite was decreasing with increase the amount of filler. So, it is concluded that the composite with 0.04% β-TCP can be used as a best composite to regenerate the bone tissue.

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