CHAPTER 1
INTRODUCTION


1.1 Overview

General introduction to nanotechnology, nanomaterials, properties, nanocomposites, methods of preparation, their applications and the aim and detail account of the present work are described in this chapter.

1.2 General Introduction to Nanotechnology

Nanoscience and technology have colossal contribution in the materials science in 21st century and have evolved as one of the most productive area for technological development. Nanotechnology can be defined as building the things from the bottom-up with atomic precision. The application of nanomaterials can be historically traced back to even before the generation of modern science and technology [1]. First defined by “Richard Feynmann”, in 1959 at a session of American Physical Society as “There is plenty of Room at the Bottom” that lead to the probability to form nanosized products with the use of atoms as building blocks. N. Taniguchi, in 1974 coined the term “nanotechnology” while E. Drexler in 1986 elaborated the concept of nanotechnological approach [2-4]. The area of nanotechnology has become one of the most fascinating fields for current research and development revolutionizing various possibilities and changing the landscape of broad range of industries such as nanoelectronics, polymer-based biomaterials, nanoparticle drug delivery, miniemulsion particles, fuel cell electrode polymer bound catalysts, layer-by-layer self-assembled polymer films, electrospun nanofibers, imprint lithography, polymer blends and nanocomposites [5]. The reinforcement aspects of nanocomposites are the crucial area of interest along with various properties and other potential applications including barrier properties, flammability resistance, membrane properties, electrical properties and polymer blend compatibilization. The synergistic advantage of nanoscale dimensions (“nano-effect”) relative to larger scale modification is the key consideration. As soon as materials are considered at nano scale, the surface area effect becomes exceptionally important which develop the concept of ‘Nanoscience’ that can be defined as the study of phenomena and application of materials at atomic, molecular and macromolecular range where properties vary noticeably from those at larger scale [6].
1.2.1 The Term “NANO”

The term “nano” refers to “dwarf” in Greek and generally used as a prefix for any unit like a second or a meter and it signify a billionth part ($10^{-9}$) of that unit such as a nanosecond is one billionth of a second and a nanometre is one billionth of a meter. We consider atom as the smallest particle while nanomaterials are considered as the smallest building blocks of nature in contemporary science. The size of proteins range from 5 to 50 nm and a hydrogen atom is 0.1 nm. Similarly, the virus ranges from 75 to 100 nm in size while the size of bacteria is about 1000 nm. Likewise, the width of DNA is about 2.5 nm and red blood cells are around 10,000 nm in diameter [Figure 1].

![Figure 1](image)

Figure 1(a): 1 nm equivalent to 10 Hydrogen atoms (b) About 2.5 nm wide- DNA molecules (c) Gold nanoparticles of about 50 nm (d) About 10,000 nm – Diameter of Biological Cells such as Red Blood Cells

1.2.2 Classification of Nanomaterials

Nanostructured materials can be created with various modulation dimensionalities such as zero (e.g. Atomic clusters, quantum dots and cluster assemblies), one (e.g. multilayers), two (e.g. ultrafine-gained over layers or buried layers/nanotubes) and three dimensional materials (e.g. bulk materials, polycrystals) Figure 2 [7]. The interest has been extended to a large variety of metals and semiconductors because nanomaterials exhibited unique properties which differ from corresponding macro-crystalline materials. Nanomaterials are classified in the following different categories on the basis of order of dimension, composition and shape.
1.2.2.1 Zero Dimensional Nanomaterials (0D)

Zero-dimensional nanomaterials are the materials in which nanoparticles are isolated from each other and comprise of nanodispersions and nanoclusters. Recently, extensive research has been devoted in the development of 0D nanomaterials such as uniform particles arrays (quantum dots), hollow spheres and nanolenses \[8\] e.g., Nanoparticles, nanoclusters.

1.2.2.2 One Dimensional Nanomaterials (1D)

Materials with one dimension in the nanometre range are generally thin films or surface coatings and include the circuitry of computer chips and the anti-reflection and hard coatings on eye glasses. The thin films have been developed by various methods and can be grown controllably finding applications in various fields such as electronics, chemistry and engineering for decades \[7\]. e.g., Nanotubes.

1.2.2.3 Two Dimensional Nanomaterials (2D)

Such nanomaterials have two dimensions in the nanometer range that include 2D nanostructured films with nanostructures firmly attached to a substrate used for separation and filtration of small particle. They owe high surface-to-volume ratios that lead to diverse mechanical and chemical properties along with broad variation in size, shape, biocompatibility, and degradability which make them suitable for a wide range of applications such as drug delivery, tissue engineering, and bio-sensing \[9\]. e.g., Films, Quantum wells.

1.2.2.4 Three Dimensional Nanomaterials (3D)

Materials in which all three dimensions are in nanometer scale are considered as 3D nanomaterials. These include thin films deposited under conditions that generate atomic-scale porosity, colloids and free nanoparticles with various morphologies \[10,7\].e.g. Polycrystals, Nanoballs etc.
1.2.3 Properties of Nanomaterials

The composites exhibiting variations in composition and structural features over a nano-range have shown remarkable melioration in properties as compared to conventional composites due to interface interactions, as given below [11]:

- High modulus
- Resistance to small molecule permeation
- Improved ablative resistance
- Increase in atomic oxygen resistance
- Impact strength retention
- Superior biological properties
- Increased thermal stability
- Improved antimicrobial property

1.3 Nanocomposites

In the current nanotechnology era, the use of building blocks with nano-dimensions makes it possible to develop new multifunctional nanocomposite materials with unique combination of properties unattainable with traditional materials. “Nanocomposites” can be defined as an exceptional class of materials having unique physico-chemical properties derived from the successful combination of the individual parent constituents into a single material in which at least one dimension of at least one component is in the nanometer size scale (< 100 nm) resulting into wide application potential in diverse fields [12]. Therefore it is necessary to endow them
with proper processability to exploit the full potential of the various applications of the nanomaterials. Nanocomposites are either synthesized in a host matrix of inorganic materials (glass, porous ceramics etc.) or by employing polymers (natural and synthetic) as one component of the nanocomposite. In 1998, Oriakhi published an article entitled ‘Nano sandwiches’ [13], stating, ‘Nature is a master chemist with incredible talent’. Nature makes strong composites such as bones, shells, and wood using natural reagents and polymers (e.g., carbohydrates, lipids and proteins) establishing exceptional examples of nanocomposites. Toyota Central Research Laboratories in the early 1990s reported that a very small amount of nano filler loading resulted in a marked improvement of thermal and mechanical properties in case of Nylon-6 nanocomposite [14], thus setting the foundation stone in this area. The properties of nanocomposite materials are dependent on the properties of their individual parents (nano filler and nylon in this case) along with their morphology and interfacial characteristics. Dramatic transitions in physico-chemical properties can be achieved with transition from microparticles to nanoparticles. In view of the fact that nanoscale materials have large surface area for a given volume, interactions at phase interfaces become largely improved resulting in enhanced properties of the material. Thus a nanostructured material can have substantially different properties from a large-dimensional material of the same composition. In the case of particles and fibers, the surface area per unit volume is inversely proportional to the material’s diameter. Therefore the surface area/volume ratio of reinforcement materials employed in the preparation of nanocomposites is crucial to comprehend their structure–property relationships. The nanocomposite materials can be classified based on their matrix materials in the following three different classes [Figure 3] [15]:

![Figure 3: Classification of nanocomposites](image-url)
1.3.1 Ceramic Matrix Nanocomposites (CMNC)

Ceramic-matrix nanocomposites have been receiving attention because of the significant enhancement in mechanical properties along with high thermal and chemical stability. The incorporation of filler components (fibres, particles etc.) in the ceramic matrix results into the increased fracture toughness and strength where the incorporated phase undergoes phase transition along with the volume expansion. The potential of ceramic matrix nanocomposites (CMNC) in case of Al$_2$O$_3$/SiC system, confirmed the apparent strengthening of the Al$_2$O$_3$ matrix after addition of a low (i.e. ~10%) volume fraction of suitable size SiC particles. Therefore it can be concluded that the incorporation of high strength nano-fibres into ceramic matrices allow the synthesis of advanced nanocomposites with high toughness and superior failure characteristics in comparison to the sudden failures as observed in case of ceramic materials [16]. Hence, in view of their superior properties, CMNC find number of uses in various fields such as optical fibres, chemical sensors etc. [Figure 4]:

e.g., Al$_2$O$_3$/SiO$_2$, SiO$_2$/Ni, Al$_2$O$_3$/TiO$_2$, Al$_2$O$_3$/SiC, Al$_2$O$_3$/CNT.

![Figure 4: Potential Uses of Ceramic Nanocomposite Systems](image)

1.3.2 Metal Matrix Nanocomposites (MMNC)

MMNC can be addressed to the materials that consist of a ductile metal or alloy matrix where some nanosized reinforcement material is implanted combining both the metal and ceramic features, i.e., ductility and toughness with elevated strength and modulus. Hence, metal matrix nanocomposites are suitable for production of materials having high strength with a great potential for application in many areas such as aerospace, automotive industries etc. [Figure 5] [17]. e.g., Fe-Cr/Al$_2$O$_3$, Ni/Al$_2$O$_3$, Co/Cr, Fe/MgO, Al/CNT, Mg/CNT.
Chapter 1

Introduction

1.3.3 Polymer matrix Nanocomposite (PMNC)

Nanotechnology has changed the face of research in polymer chemistry where polymer matrix nanocomposites have captured global interest academically as well as industrially. Broadly, polymer nanocomposites merge the two concepts, i.e., composites and nano-sized materials. The properties change significantly when the dimensions of filler particles are decreased to the nanoscale known as nano-effect [6]. In recent years the advances in synthetic techniques and the ability to characterize materials on atomic scale has led to a growing interest in nanosized materials. In contrast to the traditional fillers, nano-fillers are found to be effective even at as low as 5-wt% loading because of their dramatically higher surface area compared to their macro-size counterparts leading to tremendous interfacial contacts between the polymer and inorganic filler resulting in superior properties than those of bulk polymer phases. Thus, polymer nano-fillers composites show significantly higher modulus, thermal stability and barrier properties comparable to those of metals without much increase in the specific gravity making them commercially important [Figure 6]. Moreover, polymer nanocomposites have added advantage of lower density and ease of processability. Also, the addition of reinforcements to a wide variety of polymers produces a striking improvement in their biodegradability setting a good example of PMNC as promising systems for ecofriendly applications [18,19]. The enhanced properties of nanocomposites are strongly dependent on the particular features of nanofiller system such as its content, aspect ratio and the ratio of filler to those of the matrix as investigated from the studies and modelings using continuum mechanics. A broad spectrum of polymer features such as chemical stability, flame retardancy, scratch/wear resistance, biodegradability, mechanical, thermal, magnetic and electrical properties can be improved using nanocomposite
technology. However the dimensions and micro structure of filler phase strongly influence the final morphology and properties of the polymer nanocomposite. The wide selection of biopolymers (e.g., polynucleic acids, aliphatic polyesters, polypeptides, polysaccharides, and proteins) and fillers (e.g., metal nanoparticles, clays, and hydroxyapatite) results into the extraordinary versatility of these materials. The fundamental basis for enhanced mechanical properties relative to conventional microcomposites originate from the interaction between filler components of nanocomposites that enable them to act as molecular bridges in the polymer matrix. Therefore, these nanocomposites are of immense interest to tissue engineering, bone replacement/repair, dental applications and controlled drug delivery. Current opportunities for polymer nanocomposites in the biomedical arena arise from the multitude of applications and various functional requirements for each of these applications. For instance, the screws and rods used for internal bone fixation bring the bone surfaces in close proximity to allow healing and should be noncorrosive, nontoxic and easy to remove if required. Also, the mechanical parameters of the implant must be close to that of the bone for efficient load transfer. Thus, a polymer nanocomposite implant must meet certain design and functional criteria including mechanical properties, biocompatibility, and biodegradability. Hence, the appropriate selection of matrix polymer chemistry, filler type and matrix–filler interaction is the underlying solution to the use of polymer nanocomposites in different applications [20]. e.g., polymer/layered double hydroxides, polymer/CNT, polyester/TiO₂ and thermoplastic/thermoset polymer/layered silicates.

Figure 6: Potential uses of Polymer Nanocomposite systems
1.4 General Approaches to Nanocomposite Synthesis

Literature reveals that there are mainly two approaches under which all synthetic methods to prepare nanocomposites are being covered namely, the top-down technique which initiates with a bulk material and then breaking it into smaller fragments using mechanical, chemical or other form of energy (e.g., attrition or milling). The second approach is bottom-up wherein the nanomaterials are synthesized from atomic or molecular species via chemical reactions (e.g., colloidal dispersion) [21]. The major drawback with the conventional top-down approach is the imperfection of the surface structure as evidenced in case of ball milling and lithography methods where considerable crystallographic errors are observed which would have a significant impact on physical properties and surface chemistry of nanomaterials since the surface to volume ratio in nanostructures is very large. However, in case of the bottom-up approach, the building up of a material from the bottom: atom-by-atom, molecule-by-molecule or cluster-by-cluster is observed that plays crucial role in the field of nanomaterials research resulting into better possibility to obtain nano-architecture with less defects, more homogeneous chemical composition and better short and long range ordering.

The above mentioned approaches may be further categorized in terms of physical and chemical /wet chemical techniques/aqueous precipitation [22-24]. Broadly, physical techniques involve a process of transferring growth species from a source or target. The process proceeds from atomic state and mostly involves no chemical reactions. e.g., mechanical mixing [25]. However in case of chemical techniques, chemistry is very rich involving various types of chemical reactions e.g., direct mixing of polymer and particulate [26], solution mixing [27] etc. Chemical methods are widely used for the synthesis of nanoparticles being versatile in designing and synthesizing new materials that can be refined into the final product. The primary advantage that chemical processes offer over other methods is in achieving good chemical homogeneity involving chemical mixing at the molecular level. The wet chemical process depends on the availability of appropriate precursors. There are many chemical processes suitable for economical production in which coprecipitation is one such technique that has been used to produce nanocomposites in the present work.
1.4.1 Coprecipitation Method

Basically, the process of simultaneous precipitation of more than one compound from a solution can be defined as ‘coprecipitation’. This is the most commonly used method employed for the synthesis of various nanocomposite systems. Different processes such as mixing of solutions, nucleation, ageing, etc., are involved during the synthetic path. Initial mixing or interdispersing of components in the solution has a significant effect on the precipitation as good mixing would result in a more homogeneous product. In case of synthesis of polymer nanocomposites, the dispersion of filler in the matrix marks the most crucial step. Sonication by ultrasound with various mechano-chemical approaches can be employed for this purpose. However, re-aggregation of the individual nanoparticles limits the scope of such approaches for dispersing the nanoparticles. Polymer shell coated particles are considerably more stable against aggregation because of a large decrease in their surface energy relative to bare particles. Such a polymer shell can be obtained by first synthesizing the inorganic nanoparticles and then dispersing them in a polymer solution. Finally, the polymer-coated inorganic nanoparticles are precipitated into a non-solvating phase and the process is called ex-situ approach which is the most general as no limitations on the kinds of nanoparticles and polymers are involved [28-30]. Alternatively, the process of polymer-based nanocomposite formation and nanoparticle preparation can be performed as a series of consecutive processes in one reactor resulting into one step reaction defined as the in-situ approach in which nanocomposites are generated inside a polymer matrix by the respective precursors which are then transformed into the desired nanoparticles by suitable reactions [31-34]. The one-step synthesis leads to enhanced compatibility of the filler and polymer matrix with improved dispersion of the filler. Commonly, soluble inorganic or organo-metallic compounds are converted to colloids in water or suitable solvents by chemical reactions. The polymer may be present during the colloid formation or may be added later. On the addition of polymer, the dispersion of particle can be destabilized resulting in spontaneous formation of nanocomposites by co-precipitation or stabilized in which nanocomposites can be obtained by addition of a solvent that acts as a co-precipitation agent [35].

In the present study, both the in-situ and ex-situ approaches described above are explored. Briefly, in case of in-situ method, aqueous solutions of Ca and P precursors
are mixed in desired molar concentration under constant stirring [Figure7]. Subsequently polymer solution is added dropwise in the prior mixture with constant stirring for suitable period of time. The resulting mixture is obtained by adding a co-precipitating agent with continuous stirring at preferred temperature to obtain a homogeneous solution. The viscosity of the mixture gradually increases and precipitation initiates as soon as the pH reaches 9~11 and finally the obtained precipitate is kept for ageing at room temperature for 24 hours. After ageing, the precipitated product is filtered and washed several times with distilled water. The resulting product is either vacuum dried or calcined at around 600 °C for 4 hrs as required. The final product is crushed in the agate mortar to produce fine powder and then employed for various physico-chemical characterizations [36-38]. Similar method is followed in \textit{ex-situ} approach as described except the prior synthesis of nanoparticles followed by drop-wise addition of polymeric solution as described above.

Coprecipitation is very simple method to synthesize nanocomposites either in powder or in thin film form under mild condition. As already mentioned coprecipitation method involves wet chemical synthesis of materials so the composition of the material can be tailored at molecular level and therefore, stoichiometric control of reactants can be easily achieved. Moreover, it is cost effective because of the simple set up along with relatively lower temperature (room temp) and no delicate vacuum system needed. Also, it is possible to control the size of the particles by maintaining the pH value of the solution. Thus this approach inspires the scientific community to further investigate and modify the method to exploit its maximum value in various industrial and biomedical applications.

Figure 7: Preparation of Hydroxyapatite/polymer nanocomposite \textit{via} chemical co-precipitation pathway.
The other common methods used for nanocomposite synthesis are listed below:

1.4.2 Sol-Gel Process

Sol-gel method of synthesis comprises of multi steps involving chemical and physical processes associated with hydrolysis, polymerization, gelation, condensation, drying and densification [39]. The process initiates with the mixing of metal alkoxides or salts in a suitable solvent (usually an alcohol) or in water at ambient temperatures. The control of the pH of starting solution is crucial step to avoid precipitation as well as to form the homogenous gel that can be attained by the addition of base or acidic solutions. Organic compounds having hydrophilic functional groups (hydroxides or carboxylates) such as acrylic acid, citric acid, oxalic acid, succinic acid, tartaric acid, etc. and polymers like polyvinyl pynolidone (PVP) and polyacrylic acid (PAA) can be used with metal ion sources to form the sol as well as to control the particle size and homogeneous nature of the products [40]. Metal ions chelation by carboxylic acid groups direct to a homogeneous distribution of the constituent ions in the resulting gel which is further heated between 150 °C and 300 °C for eliminating volatile organic components, excess water etc., which results into the dried intermediate powders. The single phase nano-crystalline metal oxides are obtained after calcining dried gel powder in the temperature range of 400-800 °C depending on the precursor chemical nature. In sol-gel method, the precursors such as metal alkoxides with very high purity are needed which are not easily available commercially and thus restricting the high purity of synthesized materials.

1.4.3 Hydrothermal Process

In hydrothermal synthesis, the property of water as an excellent solvent for many ionic compounds and nonionic compounds (under high pressure and high temperature), has been efficiently exploited for the synthesis of fine powders of metal oxides. Water plays two roles as a pressure transmitting medium and solvent for the precursors. The hydrothermal conditions achieved by an autoclave brings down the activation energy for the formation of final phase, speeding up the reaction between the precursors, otherwise it would occur only at very high temperatures [41]. The pressure developed ranges between 10 to 150 kilo bar based the chosen temperature of water (>373 K).
1.4.4 Electrospinning

The Electrospinning is a versatile method that has been used to form non-woven micro- and nanofibrous scaffolds employing synthetic bio-resorbable polymers with high production capability. In this method, polymer solution is passed through a needle at a controlled rate and an electric field is applied using a high voltage source across the needle and a grounded collector charges the surface of the polymer droplet held at the needle tip. The forces of electrostatic repulsion within the solution overcome the surface tension resulting in thin polymer jet forms. As the jet travels from the needle tip to the collector, solvent evaporation occurs with the formation of polymer fibres which gets deposited on the collector. There are several variables such as solution viscosity, polymer charge density, polymer molecular weight, surface tension, electric field strength, tip-to-collector distance, needle design, and the composition and design of the collector etc. which can be varied to get desirable features and influence the process of electrospinning [42].

1.4.5 In-Situ Intercalative Polymerization

In this method layered silicates of montmorillonite type modified by cation exchange with organic ammonium salts producing organoclay is swollen within the liquid monomer and polymerization is initiated (by chemical/heat/radiation) between the intercalated sheets expanding and dispersing the clays into the polymer. An advantage of this method is the tethering effect, which allows nanoclays with chemical active surface to link polymer chain during polymerization [43]. Organophilic modification offers compatibility between filler and polymer. Such nanocomposites exhibit superior stiffness, impact, strength and heat distortion temperature.

1.4.6 Freeze Drying

Freeze-drying involves removal of a solvent (usually water) by sublimation of a frozen sample and subsequently by desorption of the non-frozen sorbed solvent under reduced pressure. Polymeric scaffolds can be synthesized by freeze drying. The polymer solution or dispersion is cooled down to a temperature in the freezing stage, where all the materials are in a frozen state resulting in the ice crystals formation, compelling the polymer molecules to aggregate into the interstitial spaces whereas the solvent is removed by applying a pressure lower than the equilibrium vapor pressure.
of the frozen solvent in the second phase. Meanwhile some of the unfrozen sorbed water in the dried layer may be desorbed. When the frozen solvent is completely sublimated by slightly warming the sample, until complete dryness is obtained. The sublimation of the ice crystals causes the formation of a highly porous sponge, the structure of which depends on the conditions during fabrication like pH, freezing rate and partial pressure. Hence, significantly porous and interconnected structures with small size pores are obtained by freeze-drying [44].

1.4.7 Solvent Casting and Particulate-Leaching

The solvent casting and particulate-leaching methodologies combination is extensively employed to synthesize 3D porous nanocomposite scaffolds. This method is based on dispersing a salt (e.g., ammonium bicarbonate, sodium chloride) in a polymer solution which is dissolved in an organic solvent. The solvent is removed leading to the solidification of the polymer followed by leaching away the salt crystals using water resulting in the formation of porous nanocomposite scaffolds. The size of the salt crystals and salt/polymer ratio controlled the size of the pore and the porosity respectively [45].

1.4.8 Direct/Melt Mixing

Direct/Melt mixing is the one of most promising method to be used in industry as solvents are not required. This method can be applied to polymer processing industries to synthesize nanocomposites on the basis of compounding devices, such as extruders or mixers. Direct mixing is employed for the fabrication of polymer silicates nanocomposite due to its simplicity, cost effectiveness and environmental friendly nature. Usually during extrusion the clay is mixed with the molten polymer that penetrates inside the clay layers inducing intercalation. Generally, high molecular weight polymers raise clay dispersion [46].

1.5 Applications of Nanocomposites

In nanotechnology, the pace of revolutionary discoveries is expected to accelerate in the next decade worldwide. This will have a profound impact on existing and emerging technologies in almost all industrial sectors including conservation of materials and energy, biomedicine and environmental sustainability. The promising technological applications of nanocomposite systems with high commercial impact, arising from different chemistries between the constituents, are abundant comprising
both the generation of new materials and the performance enhancement of known devices such as fuel cells, sensors and coatings. In view of their outstanding potentials, nanocomposites are expected to generate a great impact in world economy and business. The study of nanocomposites is an interdisciplinary area, encompassing physics, chemistry, biology, materials science and engineering [47]. Hence, the information arising from scientists with distinct backgrounds will help in the development of new materials with unforeseen technological possibilities such as designing of macroscopic engineered materials through nanoscale structures. The huge switching of nanocomposites from research to industry has already initiated and is expected to rise extensively in the coming years. Therefore, nanocomposites will be beneficial for many sectors of our society, including and chemical and electronics, space and transportation industries, as well as medicine, health care and environmental protection [48,49]. Therefore, nanocomposites may offer many advantages with improved physico-chemical properties in diverse biomedical applications involving both the performance enhancement of existing materials and generation of new materials. The huge shift from research to industry has already initiated and is expected to spread on large scale in the coming years. Brief overview of applications of nanocomposites are described below and depicted in the Figure 8.

- **Easy diagnosis and removal of tumors:** Researches are directed to unite magnetic nanoparticles and fluorescent nanoparticles in a nanocomposite to exploit their magnetic property for more visibility of the tumor during MRI procedure done prior to the surgery while their fluorescent property could better assist in locating the tumor while operating.

- **Construction of high strength-to-weight ratio structural components:** For example an epoxy containing carbon nanotubes can be used to form nanotubes-polymer composite such as windmill blades which are strong but lightweight blades that make longer windmill blades practical and thus increasing the amount of electricity generated by each windmill.

- **Synthesis of Composites with higher strength-to-weight ratios using Graphene:** Several researches showed that the addition of Graphene to epoxy composites result into stronger/stiffer components in comparison to epoxy composites using a similar weight of carbon nanotube which may be explained in
view of the fact that Graphene make a better bond to the polymers in the epoxy facilitating effective coupling leading to the manufacture of stiffer components for the uses such as windmill blades or aircraft components.

- **Fabrication of batteries with improved power output**: Researches have shown various methods to make anodes for lithium ion batteries from a composite formed with silicon nanospheres and carbon nanoparticles. The anodes made of the silicon-carbon nanocomposite allows faster charging or discharging of power by making closer contact with the lithium electrolyte.

- **Lightweight sensors using nanocomposites**: Depending upon the spacing of the nanotubes, a polymer-nanotube nanocomposite conducts electricity allowing the patches of polymer-nanotube nanocomposite to act as stress sensors on windmill blades.

- **Accelerating the healing process for broken bones**: The growth of replacement bone is accelerated when a nanofiller-polymer nanocomposite is placed as a kind of scaffold which guides growth of replacement bone as shown by many studies. The researchers are conducting studies to better understand how this nanocomposite speeds up bone growth. Nanocomposites are also expected to play crucial role in creating novel tissue regeneration strategies and in surmounting other major obstacles in tissue engineering.

![Figure 8: Overview of applications of nanocomposites](image)
1.6 Bone as a Nanocomposite System

As discussed earlier that Nanocomposites are defined as a heterogeneous combination of two or more materials where at least one material is at the nanometer scale. Nature display excellent example of a nanocomposite system in the form of bone which is a vascularized dynamic tissue serving physiological, protective and mechanical functions. Natural bone is a nanocomposite material composed of two primary phases at the nanoscale level, namely the organic phase that composed of proteins and the inorganic phase covering the mineral components along with other minor components such as non-collagenous proteins, lipids, cytokines, trace ions, water, cells etc. (Table 1). The organic phase is composed of type I collagen protein as its chief component, constituting about 30% of the total weight of the bone. The basic unit of collagen fiber, the collagen molecule, is a rod like, right-handed triple-helix approximately 300 nm long and 1.5 nm in diameter with a periodicity of about 64 nm. The organic part is stiffened by mineral component composed of hydroxyapatite (HAP/HA) \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\) needle shaped tiny crystals that account for about 70% of the total weight of the bone with dimensions of about 50 nm × 25 nm × 2 nm. The major role of mineral components is to provide toughness and rigidity to the bone whereas strength and flexibility is provided by collagen [50-51]. Therefore, understanding the contributions of nanoscale features to cell responses and bone mechanical properties remain an elusive goal to researchers attempting to create composites with mechanical properties at par with bone.

1.6.1 Bone Biology

1.6.1.1 Bone anatomy

Bone is considered as an assembly of different levels of hierarchical structural units at different scales from macro to nano to fulfill multiple functions. At the macro-structural level, a mature bone can be classified into two categories namely cortical and cancellous/trabecular bones. The cortical bone is the compact bone surrounding the marrow space and is the outer layer of most of the bones occupying various long bones with 80-90% mineralization imparting mechanical strength to the entire skeleton. Cancellous bone, conversely, is porous spongy bone that consists of 15-20% minerals and occupies the interior of bones providing structural support and organization for bone marrow interspersed inside. The cancellous bone is full of vasculature and hence
metabolic actions are taken care by it. Periosteum is the outer layer covering surface of the cortical bones mainly containing blood vessels and osteoblasts which get activated during bone repair [52]. At the microstructural level, compact bone includes osteons or harvesian systems which are the fundamental units containing cells and extracellular matrix (ECM), repeating themselves and working as weight-bearing pillars whereas spongy bone contains an interconnecting framework or trabeculae. Each osteons consists of concentric layers or lamellae approximately 3–7 mm in diameter oriented in the longitudinal direction and a central Haversian canal that contains nerve and blood supplies allowing blood and nutrient flow [53]. The osteoblasts and the mature osteocytes derived from osteoprogenitors that are located in the bone marrow and periosteum exist within the osteon contributing to the generation and maintenance of ECM giving bone its structural strength [54]. The osteoclasts derived from the myeloid are also present that mediate bone resorption. Finally, at the nanostructured level, the two major components, i.e., protein component (collagen fibers) and the mineral component (nanocrystals of HA) are found. Thus the heterogeneity, anisotropy and hierarchy of bone at the macro-, micro- and nanoscale are important contributors to its mechanical properties and biochemical properties [Figure 9].

Table 1: Composition of Bone
(Values may vary slightly on the type of bone and the species chosen)

<table>
<thead>
<tr>
<th></th>
<th>Inorganic Phase (~70%)</th>
<th></th>
<th>Organic Phase (~30%)</th>
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<tbody>
<tr>
<td><strong>Components</strong></td>
<td><strong>Wt%</strong></td>
<td><strong>Components</strong></td>
<td><strong>Wt%</strong></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>~95</td>
<td>Collagen</td>
<td>~95</td>
<td></td>
</tr>
<tr>
<td>Carbonate</td>
<td>~4</td>
<td>Water</td>
<td>~9</td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>~0.9</td>
<td>Non-Collagenous proteins</td>
<td>~5</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>~0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>~0.5</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Other traces (Cl(^{-},F^{-},K^{+},Sr^{2+}))</td>
<td></td>
<td>Other traces (Primary bone cells)</td>
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</tr>
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</table>

Also, the bone is divided histologically into immature woven bone and mature lamellar bone. In woven bone, the collagen is fine fibered oriented almost randomly. The woven bone consists of cells (osteocytes) and blood vessels. The mechanical
properties of bone depend upon the content of both mineral and protein matrix as well as the porosity, degree of crosslinking and the interaction (both physical and chemical) between the constituents. The structure and properties of bone vary according to location to fulfill the respective functions.

1.6.1.2 Bone physiology

As discussed earlier, bone is a highly dynamic tissue that undergoes constant remodelling through one's lifetime. The physiological function of bone involves hematopoiesis and mineral homeostasis. Hematopoiesis refers to the fact that bone is a repository for various ions such as calcium, chloride, potassium, carbonate, strontium, fluoride or magnesium without compromising its structure. Homeostasis is attained by the combined actions of osteoprogenitors, osteoblasts, osteocytes as well as osteoclasts. In general, bone formation is effected through the proliferation of osteoprogenitor cells and differentiation into osteoblasts which is responsible for the

Figure 9: Schematic overview of bone, depicting gross overview, and cellular distribution.
regulation of mineralization and collagen production through expression of functional proteins such as osteocalcin and alkaline phosphatase [55]. Osteoblasts entrapped in the ECM differentiate into mature osteocytes that make syncytial networks to support bone structure and metabolism. According to Wolff's law, bone actively undergoes remodelling in response to environmental stimuli such as physiologic influences or mechanical forces that involves a balance between bone formation as well as bone resorption by osteoclasts [56]. It is essential for renewing bone to maintain both bone strength and mineral homeostasis. The bone is able to undergo significant regeneration in response to trauma and fractures through the three phases of inflammation, repair and remodelling. Consequently, the repair and remodelling processes are initiated in the periosteum containing rich population of osteoprogenitors and osteocytes that proliferate and migrate to the defect site, forming a bony bridge to effect fracture healing and it is when this bridging does not happen, hypertrophic non-union occurs, that calls for the use of bone grafts. Thus, bone is an impure heterogeneous and highly substituted apatite crystal where each ion contributing to bone development and regeneration and its disparate constituents contribute unique properties to create a structure optimized not only for high loading and bending moments but also for fracture and fatigue resistance.

1.6.2 Types of bone cells

The various types of bone cells and their functions are represented in Table 2. Basically there are three types of cells that contribute to bone formation:

**Osteoblasts:** These are bone-forming cells. They secrete collagen followed by coating non-collagenous proteins similar to glue that has the ability to bind the minerals from the bloodstream, mostly calcium and phosphate.

**Osteocytes:** These are mature bone cells responsible for the maintenance of the bone tissue and function as transporting agents of minerals between bone and blood.

**Osteoclasts:** These cells resorb or break down bone. They secrete acids or enzymes to dissolve the minerals as well as collagen from the matured bone [57].
Table 2: Different types of Bone cells

<table>
<thead>
<tr>
<th>Types of Bone Cells</th>
<th>Description</th>
<th>Morphology</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblasts</td>
<td>New bone tissue forming cells.</td>
<td>Cuboidal in shape; located at the bone surface with their precursors</td>
<td>Synthesis and regulation of bone ECM deposition</td>
</tr>
<tr>
<td>Osteocytes</td>
<td>Matured cells derived from the osteoblasts</td>
<td>Stellate shaped</td>
<td>Calcification of osteoid matrix</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>Largest cells found at the surface of bone mineral.</td>
<td>Multinucleated cells</td>
<td>Bone resorption</td>
</tr>
</tbody>
</table>

1.6.3 Bone repair mechanism

Bone repair is a key process involving the interplay of complex cellular and molecular events to generate new bone instead of a fibrous scar which is the final outcome in other connective tissues. New bone formation commences by condensation of mesenchymal stem cells (MSCs) that leads to either the direct differentiation into osteoblasts at the periostium which is known as intramembranous ossification such as repair of flat cranial bones (as studied in the present research work) or the formation of a cartilage template which is subsequently replaced by bone (ossification) defined as endochondral ossification which occurs in long bones [58].

As in the present study, in case of Intramembranous mechanism of healing [Figure 10], the healing pathway initiates with immediate bleeding with disruption of the local soft tissue integrity that directs to interruption of normal vascular function distorting marrow architecture. The extravasation (bleeding) within the injured site is contained by the surrounding tissue and develops into a hematoma. The degranulating platelets, macrophages and other inflammatory cells (granulocytes, lymphocytes and monocytes) infiltrate the hematoma between the injured fragments and combat infection; secrete cytokines and growth factors and advance clotting into a fibrinous thrombus [59]. Over time, capillaries grow into the clot, which is reorganized into granulation tissue. This provide fundamental platform for an inflammatory response which involves the production of pro-inflammatory cytokines namely tumor necrosis factor-α, interleukin-1 (IL-1) and IL-6 from aggregated platelets that have chemotactic activity towards lymphocytes, monocytes-macrophages, fibroblasts and endothelial cells. Macrophages
can secrete the aforementioned cytokines and various growth factors such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), the transforming growth factor-β (TGF-β) superfamily, including bone morphogenetic proteins (BMPs) inducing angiogenesis and bone formation from recruited periosteal progenitors which is responsible for the synthesis of extracellular matrix [60]. Macrophages, giant cells and other phagocytic cells clear degenerated cells and other debris. The fundamental process for successful bone repair is the neovascularization of injured tissue that offers nutrients and oxygen and also help in metabolic waste removal. This also facilitates delivery of progenitor/mesenchymal cells of haematopoietic origin. The mechanical stability of the healing region maintains vascular integrity and elevates osteogenesis. As the process commences, the blood clot gets replaced by callus with the formation of woven bone that later replaced by lamellar bone through the coordinated activity of osteoblasts and osteoclasts resulting in the formation of mature and compact lamellar bone by the process of remodelling.

Figure 10: Schematic representation of Healing of Membranous bone (Centre: Ventral view of Mice calvarium bone: FB-Frontal Bone, PB-Parietal bone, OB-Occipital bone, DA-Defected Area)
1.7 Motivation behind the Translation of Bone engineering Concept into clinical Application: Background and Significance of the study

1.7.1 Current status of bone tissue diseases and defects

Bone defects and diseases as a consequence of trauma, injury and infections are of major concern in the field of health sector. Tissue transplantation is the second-most common procedure after blood with over 2.2 million bone graft procedures conducted worldwide annually in the realms of orthopedics and dentistry. Surgical reconstruction, transplantation (autografts and allografts), drug therapy, artificial prostheses and medical devices are the current clinical treatment options for various bone tissue related disorders. Nevertheless, these treatments have disadvantages such as severe pain, risk of infections, hematoma, immune rejection, donor site morbidity, transmission of viral (HIV, hepatitis-B) and prion proteins. However, relatively few orthopedic biomaterials designed with bio-responsive characteristics have been translated into clinical solutions to date. Approximately 600, 000 bone graft procedures are performed each year in the United States and about 2.2 million of such procedures are performed worldwide annually [61]. The global market now surrounds bone graft and bone graft substitute materials covering biomaterials, which generates in excess of $2.5 billion in sales per year. In view of the EU report on nanotechnology, the global market for ‘SMART BIO-MATERIALS’ will rise to $113 billion by 2025 [62]. The development of a biomaterial scaffold is a major aspect introducing the concept of bone tissue engineering to provide efficacious and reliable bone grafts. In this context, in recent years bone tissue engineering has emerged as an appropriate strategy to repair and/or replace damaged and/or diseased bone tissue defects.

1.7.2 Clinical need of Bone Grafts

Nature has created bone to be a composite material in several prospects i.e., being a porous material, a polymer-ceramic mixture and a fibre-matrix material that primarily composed of collagen, non-collagenous proteins, and hydroxyapatite. Therefore, its mechanical properties depend on each of these aspects of structure and composition. Generally, bone owns a high intrinsic regenerative capacity after trauma or disease and thus most of the bone defects and fractures heal without any surgical intervention. There is marked improvement in the treatment of complex fractures and defects
caused by high energy trauma, developmental deformity, disease, tumour resection, and revision surgery because of the advance surgical techniques, implant design and post-operative care. However, repair of large defects/critical size defects (size vary with species and experimental model), with limited intrinsic regenerative potential, formed as a result of extensive soft tissue injury, inadequate surgical techniques, biomechanical instability and infections have been considered as a major surgical challenge. Bone grafting has been proves to be a highly successful therapy when the bone repair mechanism fails as a result of magnitude, infection or other causes. The motivation for research and the focus on translating bone engineering concepts into clinical application is rooted in the limitations associated with the growing difficulties in orthopaedic, dental, and reconstructive surgery. The current clinically-established approaches focus on the implantation of autografts and allografts, metal devices, and ceramic-based implants to aid the repair of bone defects. But all the approaches have inherent disadvantages triggering the need for new therapeutic concepts to design and engineer unparalleled structural and functional bone grafts to replace current treatments. Therefore, the field of bone tissue engineering has emerged through the integration of engineering, life sciences, molecular and cell biology, stem cell biology, and surgery [63]. Tissue engineering can be defined as ‘the application of principles and methods of engineering and life sciences to obtain a fundamental understanding of structure-function relationships in normal and pathological mammalian tissue and the development of biological substitutes to restore, maintain, or improve tissue function’ [64]. The drive to develop bone grafts for the filling major gaps between the synthesis of suitable biomaterial and its clinical application has led to a major research thrust towards developing biomaterials for orthopaedic applications. Here in the present study, we describe the comprehensive synthesis, characterization and application of appropriate biomaterial nanocomposite systems for potential applications in bone regeneration studies *in vitro* as well *in vivo* executed in animal models keeping essential prerequisites for bone substitutes to be employed in Bone tissue engineering.

### 1.7.3 Fundamental considerations for a suitable biomaterial to be used in Bone tissue engineering

The ideal biomaterial for bone tissue engineering should have specific characteristics such as providing a momentary mechanical support to the affected area, should
possess porous interconnecting architecture for allowing vascularization and bone in-growth with the cost effectiveness of processing toward industrial scale production [65]. Further, the biomaterial should be biocompatible with appropriate surface chemistry in order to permit cell adhesion and provide sufficient integration with host bone tissue. Furthermore, a biomaterial should encourage migration of bone cell into the scaffold, should help in promoting osteogenic differentiation, osseointegration and degrade in a controlled manner to facilitate load transfer to developing bone [66]. There are several requirements of scaffold materials to meet the demands of tissue engineering as listed below:

- **Biocompatibility**: The capability to perform with an appropriate host response in a specific situation. The substrate material must not provoke an inflammatory response nor display immunogenicity or cytotoxicity.

- **Bioactivity**: The ability to form a direct chemical bond with bone and thus a uniquely strong biomaterial strong interface.

- **Osteointegration**: The healing process of implant placed within the bone

- **Osteoconduction**: The capacity to guide bone-forming tissue on a surface or down into pores.

- **Biodegradability**: The ability to gradually vanish and leave space for new tissue growth without hindering the regeneration process.

- **Osteoinduction**: The ability to induce bone formation by influencing the differentiation or maturation of stem cells into bone-forming cells remodelling of functional and highly organized bone.

- **Osteogenesis**: Osteogenesis is a formation and development process of bone initiated by connective tissue or cartilage.

- **Surface Architecture**: Suitable chemical and topographical properties for influencing cellular adhesion, proliferation and differentiation. Large surface area to volume ratio, interconnectivity, porosity, hydrophilicity allows neovascularization, mass transport and tissue in-growth.

- **Mechanical stability**: Sufficient mechanical properties to withstand *in vivo* loading forces and provide strength at the defect site. The mechanical
properties of the material must be sufficient to prevent structural failure during handling and during the patient's normal activities.

1.7.4 Current commercially available products as bone substitute materials

In particular, huge emphasis has been placed on synthetic biomaterials that encompassed bioactive properties i.e. actively interacting with surrounding bone tissue to establish a direct bond between the synthetic material and bone, osteointegration and osteoconductive properties. These advanced properties of synthetic bone substitute materials provide the basis for a progression towards the demand for more powerful biomaterials to be used for the reconstruction of bone defects and more specifically those for application in compromised clinical situations (e.g. critical-sized defect scenarios, osteoporotic patients, patients with impaired wound healing due to diabetes, etc.). In case of current commercially available bone substitute materials in modern orthopedic and dental applications, several inorganic and organic bone substitute materials, including ceramics, demineralized allografts, polymers and composites have gained popularity due to their availability, cost-effectiveness and biological performance. During the past decade, a growing range of synthetic scaffolds [Table 3] has become commercially available which are prepared in various physical forms, such as powders, granules, dense blocks, putties, pastes, gels and porous scaffolds, depending on the components and treatment for bone regeneration applications [67].

Table 3: Commonly used commercial materials for orthopedic applications

<table>
<thead>
<tr>
<th>Product</th>
<th>Material</th>
<th>Application</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-oss</td>
<td>Cancellous Bone</td>
<td>Bone grafting material</td>
<td>Geistlich</td>
</tr>
<tr>
<td>Cerabone</td>
<td>Natural Bovine</td>
<td>Bone grafting material</td>
<td>Botiss</td>
</tr>
<tr>
<td>Orthoss</td>
<td>Collagen</td>
<td>Bone graft substitute</td>
<td>Geistlich</td>
</tr>
<tr>
<td>Perioglass</td>
<td>Bone graft particulaes</td>
<td>Bone regeneration</td>
<td>Novabone</td>
</tr>
<tr>
<td>Collagen Bone graft</td>
<td>Hydroxyapatite/Calcium phosphate</td>
<td>Osseus defects</td>
<td>Zimmer/Collagen Corp.</td>
</tr>
<tr>
<td>Plexur™ P</td>
<td>Cortical fiber suspended in PLGA</td>
<td>Bone void filler</td>
<td>Osteotech</td>
</tr>
</tbody>
</table>
1.8 Biomaterials for the development of nanocomposites for Bone tissue engineering

Biomaterials have played a pivotal role in quest of achieving desirable features such as biocompatibility, suitable morphological architecture and optimum mechanical stability for the development of a competent nanocomposite system for Bone tissue engineering applications. Biomaterials that are able to mimic the structural, mechanical, and biological properties of natural bone have been captivating considerable attention in the last decade [68]. A brief description of the most promising biomaterials used in Bone tissue engineering is presented herein.

1.8.1 Polymers

Polymers are increasingly gaining interest for the development of nanocomposite scaffolds for bone tissue engineering applications in comparison to other bone substitute materials for their versatility in terms of chemical and physical properties. Polymer based-materials provide flexibility associated with a wide range of mechanical properties that make these materials to be easily fabricated and to be degraded at a controlled rate parallel to bone regeneration. Additionally, polymers can be sterilized easily without causing chemical changes and have the ability to incorporate biological matrix components. Several polymers that have been extensively studied for the synthesis of nanocomposite scaffolds for bone and regeneration applications are discussed in the following [69,70,6,42]:

1.8.1.1 Natural polymers

Natural polymers have been reported to build a better environment for cell growth, spreading and proliferation and found to be obtained from natural sources such as animal (chitosan-crustacean shells) or vegetal (cellulose-plants). Majority of these polymers are hydrophilic that make them suitable for cell culture studies. Economic and environmental aspects are contributing to the growing interest in natural polymers due to their biodegradability, low toxicity, low manufacture costs, low disposal costs and renewability. Moreover, they offer a wide range of advantages for bone regeneration applications such as biological signaling, cell adhesion, cell responsive degradation and re-modeling [71,42]. A great number of different natural polymers have been studied among which the key polymers for various bone tissue engineering applications are discussed below:
Chapter 1

Introduction

1.8.1.1.1 Chitosan

Chitosan is a biodegradable linear amino polysaccharide obtained from the deacetylation of naturally occurring polysaccharide chitin poly (β-(1-4)-N-acetyl-D-glucosamine) [Figure 11]. Chitin, a derivative of glucose is a naturally occurring long-chain polymer of an N-acetylglucosamine and the second most abundant polysaccharide found in crab, shrimp, lobster, coral, jellyfish, butterfly, and fungi employed for biomedical applications [72]. Chitosan is a cationic polymer composed of varying composition, sequence and molecular chain length of randomly distributed N-acetyl glucosamine and D-glucosamine. Hence maintain the electrostatic interaction with negatively charged biomolecules and the interaction with cell membranes. Chitosan can be developed in diverse forms like films, fibers, hydrogel and particles for various applications in bone tissue engineering and wound healing, in view of its excellent properties like biocompatibility, biodegradability, ability for cell ingrowth with intrinsic antibacterial nature that make it an important biomaterial [73]. There are several forms of pure chitosan that differ by their degrees of deacetylation (DD) and molecular weights, both of which have a strong influence on other physicochemical properties of chitosan including crystallinity, solubility, and degradation. The DD represents the ratio of glucosamine to N-acetyl-glucosamine and commonly falls in the range of 50–95%. Depending on the source and processing parameters, the molecular weight of commercially available chitosan ranges from 300 to 1000 kDa. Chitosan is insoluble in neutral and basic solutions but primary amines on deacetylated subunits of chitosan have a pKa of 6.5 and therefore chitosan forms water soluble salts in both organic and inorganic acids where free amine groups become protonated and render chitosan positively charged. The higher DD relates to a higher percentage of positively charged primary amines and therefore to higher charge density. The interesting biological properties of chitosan can be attributed to its cationic nature. For example, Chitosan binds to the negatively charged red blood cells thereby promoting clotting which results into its hemostatic property that makes it an important candidate in wound dressings. It has been shown to enhance wound healing by activating and modulating the function of inflammatory cells such as macrophages, neutrophils, endothelial cells and fibroblasts and by promoting the formation and organization of granulation tissue. Furthermore, Chitosan is
antimicrobial due to its cationic property which disrupts anions in bacterial cell walls leading to suppression of biosynthesis and disruption of mass transport across the cell walls. Chitosan gained attention as a bone scaffold material because it stimulates the attachment and proliferation of osteoblast (bone-forming cells) as well as development of mineralized bone matrix \textit{in-vitro} with non-toxic degradation products. In addition it has been shown to evoke minimal foreign body response and fibrous encapsulation when implanted \textit{in-vivo}. However, Chitosan possess low mechanical strength being unable to support load-bearing. Consequently, a diversity of materials, namely calcium phosphates (CaPs) (e.g. HAP) and variety of polymers (e.g. poly(methyl methacrylate), poly(L-lactic acid) (PLLA) etc.) has been combined with chitosan to create stronger scaffolds with superior biological and mechanical properties for bone tissue engineering [74]. By incorporating calcium phosphate into chitosan scaffold both the compressive modulus and yield strength are greatly improved. Chitosan/Hap nanocomposites have been reported to promote the formation of bone-like apatite on their surfaces after soaking in SBF and to enhance the attachment, proliferation and differentiation of osteoblast-like cells. Recently, chitosan hydrogel–Hap composite membranes were prepared through deposition of Hap on the surface of the hydrogel by a wet chemical synthesis method [75]. The studies demonstrated that the incorporation of Hap nanoparticles into chitosan fibers led to significant bone formation when compared to the bone formation onto pure electrospun chitosan scaffolds. Chemically modified HAP-chitin and HAP-chitosan composite materials were reported to be osteoinductive and exhibit rapid degradation and neovascularization \textit{in-vivo} [42]. Improved cell attachment ability is achieved by incorporation of collagen into chitosan scaffold. Hence Chitosan in view of its excellent properties finds ample and potential applications in Bone tissue engineering.

\textbf{Figure 11: Structure of chitosan}
1.8.1.1.2 Silk Fibroin

Silk fibroin (SF) is a fibrous protein that forms the filaments of native silkworm silk and displays exclusive physico-chemical properties. Silkworm silk fibers obtained from cocoons (Bombyx mori) consist of a core structural protein fibroin (70–80%), coated with sericin (20–30%) which is a water soluble glue-like protein that hold fibroin fibers together. They contain at least two major structural fibroin proteins, light and heavy chains (25 and 325 kDa, respectively) [42]. Silkworm fibroin has to be extracted from the silkworm cocoon by elimination of the sericin by boiling in an alkaline solution and the degummed silkworm silk is then dissolved in lithium bromide, dialyzed and formed in an aqueous SF solution. Bombyx mori SF has attracted increasing interest for bone, cartilage, and ligament tissue engineering because of its significant properties like elasticity, mechanical strength, biocompatibility, and biodegradability. SF is rich in β-sheet structures owing to hydrophobic domain that offer good resistance to water solubility and lead to the high mechanical properties [23]. SF can be processed into fibers, membranes, films, meshes, foams, and hydrogels for the repair of several tissues. Silk-based composite scaffolds with superior physicochemical and biological characteristics have been developed for bone tissue engineering. The incorporation of Calcium Phosphates into silk scaffolds represents an improved porous structure, osteogenic differentiation and in-vivo bone formation [76]. In addition, the addition of HAP in silk foams accelerates the osteoconductivity and mechanical properties of the scaffolds. Silk fibers have been used as sutures in biomedical applications for decades and they show slow degradability, high strength and flexibility, genetically tailorable composition and sequence, permeability to water and oxygen. Also, they can be processed in aqueous solutions into gels, sponges, powder and membranes and modified easily due to the presence of amine and acid side chains. Thus, they are attractive biomaterials for various bone tissue engineering applications [42].

1.8.1.1.3 Collagen and Gelatin

Collagen and its denatured form, gelatin are the most important extracellular matrix (ECM) proteins that show ample applications in tissue engineering because of the presence of various functional groups that accelerate osteoblast adhesion and migration. Collagen is a fibrous biological protein comprising of glycine (Gly) (~
33%) and imino acids (~20%), impart major structural and mechanical support and found as the major component of the ECM of many tissues (skin, bone, cartilage, tendons, teeth). Collagen exhibits different morphologies in different tissues, for example, (Type I) found in bone, (Type II) cartilage and (Type III) in blood vessel walls. This protein is biocompatible, non-cytotoxic, and biodegradable, but possesses low elasticity and mechanical strength. Collagen can be processed in films, fibers and foams to engineer various tissues such as bone, cartilage, heart, ligament and nerve. Collagen is also appropriate to produce scaffolds for culturing mesenchymal stem cells (MSCs) in tissue engineering [77]. It has been reported that Collagen sponges support cell and tissue attachment and growth and boost bone formation by encouraging the differentiation of osteoblasts. The nanocomposites developed from the blend of calcium phosphates (CaPs) with collagen showed increased mechanical strength compared to pure collagen in view of its excellent biological properties. This behavior has been attributed to the strong interaction between calcium-binding residues on the polymer macromolecules and the surface of the nanoparticles. The low antigenicity, biodegradable nature, and cell-binding properties of collagen make it valuable for tissue engineering applications. In parallel, Gelatin is biocompatible, non-immunogenic and bioresorbable. Although, gelatin lacks the structural characteristics of collagen but it has higher solubility and lower cost relative to collagen. Gelatin/CaPs nanocomposites with enhanced mechanical properties and better cell attachment have also been reported. The gelatin/HAP nanostructured scaffolds exhibited mechanical strength comparable to the spongy bone with an excellent capacity of cell attachment, migration and penetration into the pores of the nanocomposite. Thus, it can be concluded that both Collagen and Gelatin have been emerging as potential candidates for various bone tissue engineering applications [23,42].

1.8.1.1.4 Starch-based materials

Starch is a condensation polymer and the chief polysaccharide constituent of photosynthetic tissues and considered as one of the most promising materials because of its large availability and relatively low price combined with its inherent biodegradability and renewable origin. Starch is a biopolymer synthesized in a granular form by green plants and consists of a mixture of a linear poly(1,4-α-D-glucopyranose) (amylose) and a branched poly(1,4-α-D-glucopyranose) with branches
of (1,6-α-D-glucopyranose) (amylopectin) occurring nearly every 25 glucosidic moieties [Figure 12]. Minor constituents such as lipids, proteins, and minerals are present in starch and the levels vary with the origin. Starch is an excellent material for industrial uses due to its non-toxic, renewable and biodegradable properties. However, the intrinsic properties such as thermal, mechanical and biological properties have limited its direct applications [78]. Thus, various chemical, physical and enzymatic modifications or blending with other materials have provided solutions to achieve more desirable features. In view of its biodegradable and inexpensive nature, starch-based polymer nanocomposites find potential applications in the biomedical field such as bone cements, hydrogels for drugs controlled delivery, bone substitutes etc. Starch-based polymeric systems are commonly blended with various polymers to better resist brittleness and impart superior physico-chemical properties. The pores structure, interconnectivity and functional properties which affect the degradation behavior and mechanical properties of these materials depend on blend components, material processing technique, incorporation and nature of additives and reinforcement fillers. The presence of Hydroxyapatite as reinforcement in various starch-based materials may be helpful in the achievement of osteoblast-like cell adhesion, spreading and proliferation. The physico-chemical properties of starch-based biomaterials influence the cell proliferation rate and the presence of Hap affects the degradation behavior and the surface properties of the resulting nanocomposites [79,80].

Figure 12: Structure of Starch
1.8.1.1.5 Dates (Phoenix dactylifera)

Dates (Phoenix dactylifera) have always been a valuable crop in arid and semiarid parts of the world, being rich in several nutrients with high carbohydrate content (70–80%) offering an excellent source of rapid energy. Dates composed of a seed surrounded by a fleshy pericarp that makes about 85–90% of the fruit weight while seeds constitute about 10% of the fruit which are regarded as waste by-product and mainly used as animal feeds for cattle, sheep and poultry. The chemical composition of date seeds comprises of (% on an oven-dry weight basis): ash content (1.1±0.1); lignin (23±3.1); holocellulose (75±1.5); α-cellulose (20±1.8) and hemicelluloses (55±1.5) [81,82]. There is very little research that has been undertaken on date seeds which focused particularly on their chemical composition for nutritional purposes. Hence, there are limited studies on product development from date seeds. Some potential uses of the date seeds and their constituents in cosmetics, pharmaceuticals and to a lesser degree for food products have also been reported. In search of potential applications of this ample byproduct, there are possibilities for its conversion to materials with high additional value and products of industrial interest that can elevate the economic aspects of date fruit processing plants. The presence of compounds, with free radical scavenging activity, in the aqueous extract of date fruit impart antioxidant and antimutagenic activities. Also, the date palm seed extract is a better corrosion inhibitor for mild steel in HCl than in a H\textsubscript{2}SO\textsubscript{4} solution. There are confined nutritional, chemical and bioactivity studies available on date fruits and continuous efforts have been made to explore the chemical constituents and biological properties of date seeds in the recent years [83].

1.8.1.2 Synthetic polymers

Synthetic polymers have been emerging as attractive candidates with increased applications as they exhibit predictable and reproducible physical, chemical and degradation properties that can be modified to meet the specific requirements of different applications. However, they lack the biological hints inherent in many natural materials that encourage desirable cell responses. Synthetic polymers have a major drawback of immunogenicity, infections and toxic degradation byproducts. However the effects can be minimized by incorporating them with natural polymers/ceramics/metals etc., balancing the resulting outcomes and hence
broadening the range of properties of this class of materials [84]. The most commonly used synthetic polymers are listed below:

1.8.1.2.1 Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides comprising of a family of compounds which is made up of α-D-glucopyranoside units bound via α-1,4-glycosidic linkages together in a ring having the shape of a hollow truncated cone, capable of forming inclusion complexes with various guest molecules in the solid state as well as in solution with sizes compatible with the dimensions of the cavity. They are also known as cycloamyloses and cyclomaltoses. They are formed as a result of intramolecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme which is naturally excreted by Bacillus macerans, yielding a mixture of six-, seven- and eight-member rings parallel to α-CD, β-CD and γ-CD, respectively [85]. CDs possess similar physico-chemical and biological properties as their analogous water-soluble linear dextrins where the α-acetal linkages (i.e., the glycosidic bonds) of the terminal glucose units are hydrolyzed faster comparative to non-terminal units [Figure 13]. In view of their cyclic structure, CDs are three to five times more resistant to non-enzymatic hydrolysis relative to linear dextrins. CDs are as stable as sucrose or starch in the solid state and can be stored for several years at room temperature without any noticeable degradation. The α-CD is approximately 1.5-times more stable and γ-CD is approximately 1.5-times less stable than β-CD. Cyclodextrins are the most accessible, the lowest-priced and generally the most useful and thus produced globally on an industrial scale. The important characteristic of cyclodextrins is the formation of inclusion compounds in both the solid and solution states where each guest molecule is surrounded by the hydrophobic environment of the cyclodextrin cavity leading to changes in physical, chemical, and biological parameters of guest molecules, eventually resulting in considerable pharmaceutical potential enhancing its chemical stability [86]. Many cyclodextrin derivatives have been synthesized by aminations, esterifications or etherifications of primary and secondary hydroxyl groups of the cyclodextrins, apart from naturally occurring cyclodextrins. The solubility of the cyclodextrin derivatives is usually different from that of their parent cyclodextrins depending on the substituent. Keeping in view of their ability to link covalently or non-covalently specifically to other cyclodextrins, cyclodextrins can be used as
building blocks for the construction of supramolecular complexes. Therefore, molecular architectures such as catenanes, rotaxanes, polyrotaxanes and tubes can be constructed.

Figure 13: Structure of β-cyclodextrin

1.8.1.2.2 Polyethylene glycol (PEG)

Polyethylene glycol (PEG) is a polyether compound also known as polyethylene oxide (PEO) or polyoxyethylene (POE). The structure of PEG [Figure 14] is commonly represented as H−(O−CH₂−CH₂)ᵅ−OH where n is the average number of repeating oxyethylene groups ranging nearly from 4 to 180 having two hydroxyl ends, which can be converted into other functional groups such as methyloxyl, carboxyl, amine, thiol, azide, vinyl sulfone, acetylene, and acrylate.

Figure 14: Structure of PEG

Polyethylene glycol is biocompatible, neutral, non-toxic, odorless, lubricating and non-volatile polymer group of biomaterials and found applications in a variety of pharmaceuticals and in medications as a solvent, dispensing agent, ointment and
tablet excipients in view of its segmented block co-polymer character. In addition, PEG is non-immunogenic, non-antigenic, and protein resistant polymer and has been used in several FDA-approved products. The PEG coating could raise the compatibility between nanoparticles and aqueous medium preventing particle surface from oxidation, reduce toxicity, and facilitate storage or transport [87]. PEG has been an important type of hydrophilic polymers owing outstanding physical–chemical and biological properties including surface modification, bioconjugation, drug delivery and tissue engineering [88]. PEG is soluble in both water and organic solvents and thus easy for chemical modifications. PEGs of different molecular weights have attracted intensive interest in drug delivery systems and bone tissue engineering. It is reported that when PEGs with different molecular weights are introduced into in-vivo systems, they depart from the drug delivery vehicles and may participate into biomineralization. Therefore, investigation of the effects of PEGs on Hydroxyapatite (HAP) crystal growth might give some clues to the understanding of the interactions between PEG implants and natural tissues [89]. Moreover, they find application as plasticizer to increase lubricity and to impart a humectant property in ceramic mass, adhesives and binders. The wide range of chain lengths provides identical physical and chemical properties of versatility which is utilized in terms of tailoring their applications, such as tissue scaffolding, artificial cartilage and biodegradable scaffolds.

1.8.1.2.3 Polyurethane

Polyurethanes (PUs) are emerging as a unique class of biomaterials due to flexibility associated with their versatile chemistry which is responsible for tailoring scaffolds in order to attain desirable chemical, physical and mechanical properties such as durability, elasticity and fatigue resistance by altering the ratio of the starting materials [90]. PUs comprises large family of polymeric materials with an enormous diversity of chemical compositions, mechanical properties, tissue-specific biocompatibility and biodegradability. PUs are commonly synthesized using three components: a diisocyanate, a polyol, and a chain extender (usually a diamine or diol) through step growth polymerization. The resulting polyurethanes are phase-segregated polymers consisting of alternate polydispersed blocks of ‘soft’ segments (made of macropolyols) and ‘hard’ segments (made of diisocyanates and chain extenders). The segmented PU elastomers can undergo microphase separation to build
hard and soft domains due to differences in polarity between the hard (polar) and soft (non-polar) segments. The soft domains are rubbery and amorphous at room temperature owing the glass transition temperature of less than 0 °C. The hard domains that result in the induction of hydrogen bonding between urethane and urea groups in the hard segments of adjacent polymer chains function as physical cross-links that inhibit flow when stress is applied to the materials. The biodegradation rate and mechanical properties can be altered by modifying the structure of the hard and soft segments and/or changing the relative ratios of the hard and soft segments. The soft segments generally dominated the degradation behavior of PUs and a high fraction of soft segments tends to enhance the degradation rate. Recently many attempts have been made to raise the biodegradability of PUs. Many researchers have been utilizing the flexible chemistry of PU materials to design degradable polymers for tissue engineering, including both hard and soft types. Generally, PUs are recognized to have good blood and tissue compatibility. PUs when combined with lysine diisocyanate (LDI) displayed no considerably detrimental effects on cell viability, growth, and proliferation *in-vitro* and *in-vivo*. The LDI-based PUs when implanted subcutaneously in rats revealed that they did not aggravate capsule formation, accumulation of macrophages, or tissue necrosis. The PU elastomers also have a superior processability in addition to their tunable mechanical and biodegradable properties and hence fabricated into highly porous scaffolds by a number of foaming techniques, such as thermally induced phase separation, salt leaching/freeze-dry and electrospinning. Using suitable fabrication method, different porosities, surface-to-volume ratios, and three-dimensional structures with concomitant changes in mechanical properties can be attained for wide range of tissue engineering applications including bone and soft tissues. In addition, the biocompatible and biodegradable polyurethanes have been investigated as scaffolds for tissue engineering applications and also as heart valves, stents, intra-aortic balloons and pacing lead insulators [91].

### 1.8.1.2.4 Phenol formaldehyde resins (PFRs)

Phenol formaldehyde resins are the synthetic polymers prepared from phenol and formaldehyde and extensively employed as commodity and engineered materials in the high technology transportation industry. Phenolic resins can be synthesized under both acidic and alkaline conditions. The acid-catalyzed resins called Novolacs which
are prepared at a formaldehyde/phenol ratio between 0.75 and 0.85, while alkaline-catalyzed resins called Resoles are prepared at a formaldehyde/phenol ratio higher than 1 by step-growth polymerization reaction. The phenol units are mainly linked by methylene and/or ether groups. The reactive species is the phenoxide anion \((C_6H_5O^-)\) formed by deprotonation of phenol. The negative charge is delocalized over the aromatic ring, activating sites 2, 4 and 6, which then react with the formaldehyde. Being thermosets, hydroxymethyl phenols will crosslink on heating to around 120 °C to form methylene and methyl ether bridges through the elimination of water molecules. The high crosslinking is responsible for its hardness, good thermal stability, and chemical imperviousness [92]. The phenolic resins retain industrial and commercial interest despite of the emergence of several superior classes of thermosets, high-performance polymers and several other new generation materials because they possess numerous desirable characteristics such as superior mechanical strength, heat resistance and dimensional stability as well as high resistance against various solvents, acids and water. The phenolic resin composites find usage in thermo-structural applications in the aerospace and railway industries because of their good heat and flame resistance, excellent ablative features and low cost. As a result of innovative research, new phenolic products and applications persist to emerge displaying their versatility and the capability to cope up with the challenges of advanced technology. Resols are major polymeric resin materials widely used for gluing and bonding building materials [Figure 15]. Exterior plywood, oriented strand boards (OSB), engineered laminated composite lumber (LCL) are typical applications. Nevertheless, phenolic resin nanocomposites are exceptional materials that have attracted considerable attention in both scientific and industrial fields on account of their unexpected hybrid properties and thus have been used for industrial applications, such as structural adhesives, coatings, moulding compounds, thermal insulation materials and tissue engineering due to their low-cost, easily processability, high chemical resistance, fine dimensional stability, good temperature resistance and superior mechanical strength properties [93].
Poly-α-hydroxy esters such as poly(lactic acid), PLA, poly(glycolic acid) PGA poly(lactic acid-co-glycolic acid) PLGA are a family of linear aliphatic polyesters that are the most common and widely used synthetic polymeric materials in bone tissue engineering. They have been found to be biocompatible and degrade into non-toxic products with a controllable in-vivo degradation rate and thus employed as degradable surgical sutures [94]. The ester bonds in these polymers are hydrolytically labile and these polymers degrade by non-enzymatic hydrolysis. The degradation products of PGA, PLA and PLGA are nontoxic, natural metabolites, and are eventually eradicated from the body in the form of carbon dioxide and water. The degradation rates of these polymers can be tailored by altering the chemical composition, crystallinity and molecular weight. PGA is hydrophilic in nature and can be processed into non-woven fibrous fabrics and thus it is the most widely used scaffolds in tissue engineering. However compared to PGA, the extra methyl group in the PLA repeating unit makes it relatively more hydrophobic, thus reducing the molecular affinity to water that leads to a slower hydrolysis rate. The morphology and crystallinity strongly influence PLA rate of biodegradation and mechanical properties, thus PLA scaffold degrades slowly in-vitro and in-vivo, maintaining mechanical integrity until several months in comparison to PGA that degrades swiftly in-vivo and loses mechanical integrity within few weeks. Therefore, to attain intermediate degradation rates between PGA and PLA, various lactic and glycolic acid ratios are used to synthesize PLGA. PLGA
copolymers with different PGA/PLA ratio (50:50, 65:35, 75:25, 85:15, 90:10) are currently applied in tissue regeneration and generally for suture applications [95]. These polymers (PLA, PGA, and PLGA) are among those few synthetic polymers that have been approved by the FDA (US Food and Drug Administration) for certain human clinical applications and thus emerging as potential candidates for bone tissue engineering.

1.8.1.2.6 Poly(propylene fumarate)

Poly(propylene fumarate) (PPF) is an unsaturated linear copolyester that holds two ester bonds and one unsaturated carbon-carbon double bond as repeating units. The double bonds allow PPF to be cross-linked into a polymeric network via thermal treatment or using a photoinitiator. PPF forms the biocompatible and biodegradable products (mainly propylene glycol and fumaric acid) that are readily removed from the body similar to PLA and PGA on hydrolysis of its ester linkages. The double bond along the backbone of the polymer allows cross-linking in-situ causing a moldable composite to harden within few minutes. The alterations in molecular weight lead to the variations in mechanical properties and degradation time of the composite. Consequently, the preservation of the double bonds and control of molecular weight during PPF synthesis are decisive issues. However, PPF is liquid at room temperature making it difficult to manage and its low mechanical strength in view of bendy C–O–C regions in the polymer backbone limits its applications. Therefore to expand its potential uses, PPF can be combined with other polymers, fillers and ceramics that have been reported to be suitable for bone replacement owing to the outstanding biocompatibility, osteoconductivity and ability to promote cellular functions [96]. In view of the aforementioned properties, PPF have already been widely used in bone tissue engineering, although there are ongoing research efforts in improving the functionality of these polymers to further expand their applications.

1.8.2 Metal nanoparticles

Metal Nanoparticles have been studied with growing interest as they show significantly unique physical, chemical and biological properties from their bulk counterparts. The shape and size strongly influence the electromagnetic, optical and catalytic properties of metal nanoparticles such as silver (Ag), Titanium (Ti), Zinc (Zn), etc. that encouraged an expansion in research on the synthetic routes permitting
better control of shape and size. Recently, metal-based nanoconjugates are used in various biomedical applications such as probes for electron microscopy to visualize cellular components, drug delivery, detection, diagnosis and therapy (targeted and non-targeted). There is a very strong concern for the use of metal nanoparticles because of the surface effects and the dramatic changes in properties occurring when dimensions are made to nano levels. The embedding of nanoscale metal structures into polymeric matrices represents the simplest way to take advantage of their physical characteristics along with the superior biological features offered by polymer counterpart. Apart from the biological roles, metallic ions are also cost effective, stable, safer and less likely to induce strong immune response than other products like recombinant proteins [95]. Among this class, one of the most widely used metal nanoparticles includes Silver (Ag) nanoparticles that have been known to have a disinfecting effect and have aptly been investigated for their antibacterial property for various medicinal applications. Considerable attempts have been made to investigate this property which revealed size-dependent interaction of silver nano-particles with bacteria that release silver ions in a controlled manner leading to a strong antibacterial activity against a large number of bacteria. It has been reported that the nanostructured silver materials increases the inhibitory capacity because nanostructured materials have a high surface area to contact. In addition to its antimicrobial action, Ag nanoparticles promote wound healing, reduce scar formation, and diminish inflammation. However, they are easily aggregated because of high surface free energy and thus can be oxidized or contaminated in air which limits their use. Therefore, blending nano-sized metal into biodegradable polymer matrices provide a solution to these stabilization problems and allow controlled antibacterial effect. The low concentrations of silver nanoparticles also induce surface morphological changes in the polymer matrix affecting surface nanocomposite wettability and roughness that influence the bacterial adhesion process on the nanocomposite surface and thus find suitable applications in Bone tissue engineering [97]. Further, another emerging candidate in this class includes the Titanium dioxide (TiO$_2$) nanoparticles which are both environmental friendly and biocompatible exhibiting suitable cell viability in-vitro and in-vivo indicating good potential to be a biomaterial. Titanium (Ti) is a highly lustrous transition metal with low density, high strength, high fracture toughness, biocompatibility and resistance to corrosion. Titanium has been employed as implant for hard tissue replacement in view of its
lightness and good mechano-chemical properties, the major features for dental and orthopedic implant applications for decades. It is reported that the TiO$_2$ nanoparticles and calcium-based bioceramics chemically bond to living bone by inducing the nucleation and growth of bioactive apatite layer through biomineralization. In addition, TiO$_2$ nanoparticles have been proven to be toxic against malignant bone tumors, namely osteosarcoma and chondrosarcoma. Hence, there are good possibilities for a TiO$_2$-based biomaterial that provides a combination of local drug delivery and bioactivity to be used for tissue engineering applications [98]. Another most explored nanoparticle under this class include Zinc (Zn) which is a natural ingredient of human bone with percentage of 0.012 to 0.025 by weight, having high antibacterial activity, low toxicity, chemical stability and thermal resistance. The Zn nanoparticles increase bone ALP activity in rat calvarium in vitro as well as in-vivo. It is found that Zn ions have direct specific proliferative effects on osteoblasts and an effective inhibitory effect on osteoclasts in-vitro. The Zn nanoparticles have an excellent antimicrobial property against both gram-positive and gram-negative bacteria as well as fungi. It is suggested that Zn could play an important role in osteoblast mineralization along with the stimulation of collagen production in rat calvarium and thus promotes bone formation [99]. Thus in view of the aforementioned features, the metallic nanoparticles appears to grow as a broad class of biomaterials for the various bone tissue engineering applications.

1.8.3 Calcium phosphates based materials (CPCs)

Calcium phosphate ceramics (CPCs) is a class of tunable bioactive materials that have been widely used for bone tissue repair due to their similarity with the inorganic part of major normal calcified tissues of mammals (bones, teeth and antlers). The CPCs possess surface properties that support osteoblast adhesion/proliferation (osteoonduction) and stimulate new bone formation (osteoinduction). More significantly, CPCs have been shown to promote bone growth and recruit bone marrow stromal cells (BMSCs) to ectopic sites to induce bone formation [100]. The main attractive feature of these materials is their ability to form a strong direct bond with the host tissue resulting in a strong interface. The application of ceramics is frequent in maxillofacial, dental and orthopaedic surgery for filling and coating implant surface of host bone. The important CPCs such as hydroxyapatite (HAP/HA), tri-calcium phosphates (TCPs), amorphous calcium phosphates (ACP) and biphasic
calcium phosphates (BCPs) which possess remarkable biocompatibility, osteoconductivity and bioresorbability and have been extensively studied to develop nanocomposites for bone tissue regeneration are given below:

1.8.3.1 Hydroxyapatite (HAP/HA)

Hydroxyapatite (Ca\(_{10}(\text{PO}_4)_6(\text{OH})_2\)) is a fascinating and most promising biomaterial having chemical and structural features similar to the mineral component of bone (70% HAP), enamel and dentin showing excellent biocompatibility, bioactivity and osteoconductivity and thus has earned significant interest for potential reconstructive and prosthetic materials for bone, teeth and maxillofacial applications. Among the various monophasic CPCs, HAP is the most stable and least soluble phase characterized by a Ca/P ratio of 1.67 providing nucleating sites for the precipitation of apatite crystals in culture medium [101]. HAP is thermodynamically stable at physiological pH. Its porous character has ability to promote much faster tissue growth into the pores and offers high binding affinity for a variety of pharmacological substances such as antibiotics, hormones, enzymes, antibody fragments etc. Generally the size and morphology of the HAP particles have a direct influence on their ability to reinforce materials. There is extensive research on the preparation of bone substitutes to increase the structural rigidity by synthesizing HAP at nanoscale termed as nano-hydroxyapatite (n-HAP/n-HA) using wet-chemical processes for biomedical applications. The n-HAP resembles the apatite component of natural bone that is embedded between the collagen fibers having needle like morphology with 2-10 nm thickness, 15 to 30 nm width and 30 to 50 nm length warranting extremely large specific surface-to-bulk ratio [102]. The n-HA is highly osteoconductive exhibiting bone bonding capacity that promotes cellular functioning, allowing the expression of bone forming osteogenic markers [103]. Despite its encouraging biological properties, the low mechanical properties of n-HA have hindered its clinical applications. However, this limitation can be overcome by incorporating n-HA with other biomaterials like polymers, such as chitosan and also by integrating it with one-dimensional (1D) nanostructures including nanorods, nanofibers or nanotubes to provide the mechanical properties required for an implant in the reconstruction and regeneration of bone tissue. In addition, the physico-chemical and biological properties can be enhanced via ionic substitutions. For example, anionic substitution of carbonate for phosphate has been shown to increase HAP bioactivity and solubility.
while stability got raised by substitution of fluoride for hydroxide resulting in Fluoro-
hydroxyapatite formation [104]. Cationic substitutions such as magnesium in place of
calcium can potentially have favorable biological effects. HAP may be employed in
forms such as powders, porous blocks or beads to fill bone defects or voids. The bone
filler will provide a scaffold and encourage the rapid filling of the void by naturally
forming bone providing an alternative to bone grafts. HAP with various morphologies
and surface properties have also been investigated as drug carriers for the delivery of
a variety of pharmaceutical molecules because of their biocompatible, osteoconductive, non-toxic and non-inflammatory properties. Therefore, it is
interesting to investigate the broad spectrum of various systems involving
combination of biopolymer and HAP (polymer-ceramic nanocomposites) that can
provide suitable platform with enhanced cell supportive property and mechanical
strength for bone tissue engineering.

1.8.3.2 Tricalcium Phosphates (TCP)

Stoichiometric TCP \((\text{Ca}_3\text{(PO}_4\text{)}_2)\) possesses a Ca/P ratio of 1.5 and occurs in two
polymorphic forms, \(\alpha\) and \(\beta\) possessing identical chemistries but different crystal
structures which are less stable than HAP and hence more soluble in aqueous
environments [100]. \(\beta\)-TCP is considered to be both osteoconductive and
osteoinductive and thus extensively been used in bone regeneration while \(\alpha\)-TCP is
biodegradable and it is used in bone voids. \(\beta\)-TCP is bioresorbable because of its
greater solubility than HAP in physiological environments and thus successfully used
to correct periodontal defects and to augment bony contours. When TCP and
Tetracalcium phosphate (TTCP, \(\text{Ca}_4\text{O(PO}_4\text{)}_2\)) are implanted, they degrade gradually,
totally replaced by the host tissue. TCP has been used as a ceramic bone substitute
material in the orthopedic field, craniofacial surgery and also as a filling material in
reconstructive surgery [105].

1.8.3.3 Amorphous calcium phosphates (ACPs)

ACPs are a family of CPCs characterized by a lack of long-range order and can be
produced by rapid precipitation of calcium and phosphate ions from aqueous
solutions. They are deficient of a well-defined stoichiometry and marked by the
existence of impurities (e.g. \(\text{Na}^+\), \(\text{Cl}^-\)). ACPs exhibit a broad range of Ca/P ratios
(ranging between 1.15-1.67) depending on synthesis conditions such as pH and
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starting ratio of ions in solution [106]. Their amorphous nature, low Ca/P ratio and high concentration of ionic substitutions (e.g. Na\(^+\), K\(^+\), Mg\(^{2+}\), CO\(_3^{2-}\)) impart a high degree of solubility. Furthermore, their ability to release calcium, phosphate and other ions in aqueous environments contribute towards their osteoinductivity that make them an eligible candidate to be used for orthopedic applications [100].

1.8.3.4 Biphasic calcium phosphates (BCPs)

BCPs are a family of two-phase ceramics that unite the low solubility and osteoconductivity of apatite with the osteoinductivity of a more soluble phase such as TCP. BCPs may be produced physically by mixing HAP and TCP or chemically by sintering calcium-deficient apatites at high temperatures resulting in a mixture of two different phases [107]. Specific chemical properties of BCPs such as solubility depend greatly upon the characteristics of the individual phases and their relative amounts. The Ca/P ratios of BCPs typically fall between those for pure TCP and HAP.

1.9 Nanocomposites in orthopaedic applications: an insight into Bionanocomposites

The interaction of a material with bone cells is an essential property for the development of bone substitute materials as cellular processes play vital roles in the construction of the hierarchical nanocomposite structure mimicking natural bone. Nanostructured biomaterials particularly nanocomposites are perceived to be favorable and potentially supreme for bone tissue engineering applications. These materials assist bone cell growth and subsequent tissue formation in view of their nanoscale functional characteristics [53]. Synthesis of a capable nanocomposite system with desirable properties such as optimum degradation rate, bioresorbability, non-immunogenicity, interconnected and optimum porosity, high mechanical strength, biocompatibility and easy manufacturing process are one of the most challenging tasks in the area of bone tissue engineering [23]. Nanocomposites mimic the hierarchical, nanoscale composite structure of bone and thus emerge as a viable class of materials. The incorporation of nano-sized topography in the composites leads to nano-scale characteristics. The nanophase ceramics, metals and polymers support increased bone cell function in comparison to their microstructured counterparts. Nanotopography has been shown to enhance cell spreading and production of osteocalcin and osteopontin, which are the markers of osteoblast differentiation specifically for polymers [108,95]. Nanophase ceramic reinforcement into natural and synthetic polymeric scaffolds has shown to
increase strength and compressive modulus. As an emerging and fascinating class, “Bionanocomposite” has become a widespread term to design nanocomposites incorporating a naturally occurring polymer in combination with an inorganic moiety and showing at least one dimension on the nanometer scale. This brings together biology, materials science and nanotechnology, resulting in enhanced properties in that they are biocompatible and biodegradable. Bionanocomposites use a combination of several biocompatible materials and bone minerals which structurally tuned to resemble the natural bone and thus become emerging and promising artificial bone materials. They are derived from natural and synthetic biodegradable polymers such as aliphatic polyesters polysaccharides, proteins, polypeptides and polynucleic acids and organic/inorganic fillers such as hydroxyapatite, clays, and metal nano-particles. These nanocomposites offer larger surface area, high surface reactivity, relatively strong interfacial bonding and better design flexibility compared with conventional composites and thus offering the probability of creating bone grafts using bone tissue engineering processes that involve the effective applications of bone or stem cells, scaffolding and cell-scaffold interactions [20]. An ideal bone-mimetic bionanocomposite would replicate the predominant co-alignment of the organic and mineral phase. The other consideration being to employ calcium-rich bone minerals along with sufficient phosphorus to maintain the required bone mineral density (BMD). Furthermore, one of the major problems in the current implants is the low oxygen supply which causes poor bone cell proliferation. The cells of bone receive oxygen from limited sources (the nutrient artery being the major one). Hence, it is crucial that the bionanocomposite materials have sufficiently high elemental oxygen available that can favor revascularization for nutrient and compensate for the loss of oxygen delivered to cells. Bionanocomposites based on inorganic solids with a layered arrangement (1D nanoscale materials) are of singular magnitude because of their functional properties. Materials scientists are making huge efforts in such nanohybrids research because of the excellent features derived from natural polymers (improved mechanical properties, higher thermal stability and gas-barrier properties) with remarkable advantages of biocompatibility and biodegradability that results in interesting biomedical applications. Nacre in pearls and shells, ivory, bones and enamel and dentine in teeth are fine examples of bionanocomposites found in Nature. Taking into account their common properties, bionanocomposites can be integrated in a new field at the frontier of materials science, life sciences, and nanotechnology. Two main reasons have propelled
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the use of biopolymers in the synthesis of nanocomposites, replacing the commonly employed petroleum-derived polymers. The first one is related to the biodegradability of the materials resulting from the incorporation of these natural polymers, this property being decisive for the development of environmental friendly materials that help to reduce the pollution caused by plastic waste. On the other hand, biocompatibility is a crucial property for the application of these biohybrids in food packaging or tissue engineering in regenerative medicine. The future development of novel bionanocomposites with improved properties and multifunctionality can be envisaged as an emerging open field of research with plenty of possibilities because of the great abundance and diversity of biopolymers in nature as well as the advantage of their synergistic combination with inorganic nanosized solids [109,110].

1.10 Conclusion and future outlook of polymer nanocomposites in Bone tissue engineering

The introduction of polymer nanocomposites in the field of bone tissue engineering allows mimicking of the complex architecture of native bone tissue providing an innovative and practical approach to the massive production of materials for bone tissue engineering. Natural or Synthetic polymer matrices contribute a wide range of mechanical properties and exhibit suitable physico-chemical features, whereas various inorganic nanoparticles offer the fundamental bioactivity. Therefore, their integration provides the possibility to produce nanomaterials that imitate the structural and morphological architecture of native bone. Therefore, the focus on the development of polymer nanocomposites for bone tissue regeneration applications should be directed on the combination of appropriate mechanical properties with excellent biocompatibility and suitable biodegradation rate. Notably, Polymer nanocomposites based on the blending of nano-hydroxyapatite with biocompatible polymers have displayed the promising feasibility and potential for bone tissue regeneration. However, for the optimal bone tissue regeneration and their clinical applications, significant research attempts are required in terms of designing of material, composition–structure properties relationships and the biological responses to optimize the composition, structure and properties of various polymer nanocomposites for the better understanding of the actual mechanism of nanocomposite–tissue interactions in order to finally acquire the full potential of nanocomposites in bone tissue engineering.
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