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# Alginate in Bone Tissue Engineering

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## INTRODUCTION

Bone fracture resulting nonunion defects become a major concern in orthopedic care. Recent survey revealed millions of patients avail bone defect repair globally in a year and is expected to double by 2020. The demand for subsequent replacement of bone has opened up a thrust interest to find out suitable solution. Even though tissue engineering is a viable option, repairing of bone tissues is still a challenge because these hard tissues possess inherent reluctance to grow by it. When artificial scaffolds made up of polymer-based composites are employed in the defect site, it gives unique physiological and biological conditions to enhance the bone regeneration process (Gupta et al., 2016; Gogoi et al., 2017). The advantages of utilising polymeric materials in tissue engineering include tunable properties like mechanical strength, porosity, microstructure, degradability, size, shape, wettability, surface morphology and electrical conductivity (Svorcik et al., 2001; Slepicka et al., 2015; Gogoi et al., 2017). There are number of synthetic and natural polymeric scaffolds with these properties. Some of them are alginate, amylopectin (Rajesh and Ravichandran, 2015), chitin (Madhumathi et al., 2009), chitosan (Rajesh et al., 2012; Shahriarpanah and Nourmohammadi, 2016), collagen (Cao et al., 2015), gellan (Rajesh et al., 2016), silk (Correia et al., 2012), and polyesters (Idris et al., 2010).

Alginate is a naturally occurring polysaccharide isolated from brown sea algae and a linear block copolymer of (1–4)-linked-D-mannuronic acid (M units) and (1–4)-linked-L-guluronic acid (G units) residues. It forms stable cross linked structure with divalent ions like  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$ , and  $\text{Ba}^{2+}$  through ionic interactions with carboxyl functional group of G units and produced egg box structure with the negatively charged functional group present in the

G units (Lin and Yeh 2004). Alginate is used in food and beverage industry as a thickening, gelling and colloidal stabilizer because of its availability, biocompatibility and tunable properties (Gombotz and Wee 2012). Further, the bioadhesive property possessed by alginate made them to be potent material in several biomedical applications including BTE (Alsberg et al., 2001), dental impression (Nandini et al., 2008), wound dressing (Balakrishnan et al., 2005), drug delivery (Tonnesen and Karlsen, 2002), protein delivery (George and Abraham, 2006), and cell immobilization (Kong et al., 2003). The bioadhesive property helps to adhere or interact between two different surfaces (polymer and biological), so that cells can easily adhere and proliferate on them (Gombotz and Wee 2012). Moreover, the hydrophilic nature of alginate facilitates the interactions with cells and provide micro-environment for the diffusion of nutrient molecules (Lee and Mooney 2012; Khavari et al., 2016). Hence, alginate is used in BTE for the efficient growth of osteoblast cells.

## BONE

Bone, a metabolically active nanoscale composite possesses unique abilities to self-heal, and remodels itself without leaving any scar (Ghaee et al., 2017). The strength of bone is influenced by several factors including density, microstructure, and geometry of cortical or compact (around 80% of total skeleton) and cancelous or trabecular bone (around 20% of the total skeleton). Cortical bone is hard and has 10% porosity, which can be divided into long bones (femur and tibia), short bones (wrist and ankle) and flat bones (skull vault and irregular bones). Cancelous bone is sponge-like with higher porosity of about 50%–90% commonly found in metaphysis of long bones and in the vertebra of the bodies. The interaction of osteoblast, osteocyte, and osteoclast cells plays crucial role in elaboration, maintenance and resorption of this type of bone. The complex architecture of bone is composed of organic (20%) and inorganic (70%) materials along with water (10%). The organic materials of a bone comprise an extracellular matrix (ECM), which is composed of collagen, osteocalcin, osteonectin, osteoadherin, fibronectin, thrombospondin, proteoglycan, and glycosaminoglycan. Alternatively, the major inorganic part of a bone is hydroxyapatite (HAP) in addition to a few more minerals including magnesium, potassium, fluoride, phosphate and citrate.

Bone plays a major role in several essential processes and provides structural support for the body. It possesses the ability to rapidly mobilize the mineral stored based on metabolic demand. Further, it acts as a mineral reservoir. It support muscular contraction resulting in motion, which in turn help to withstand load and protects internal organs. Furthermore, the inner spaces of bone

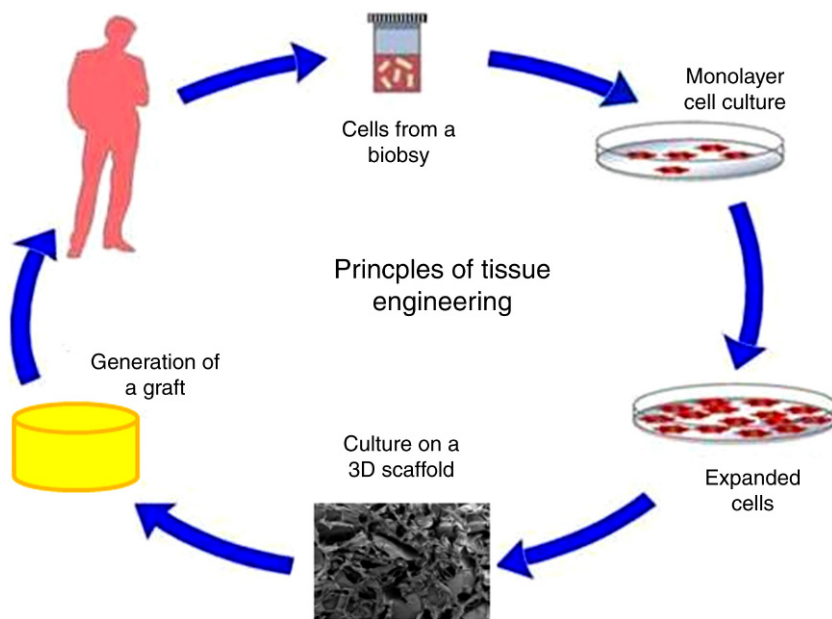
provide an appropriate microenvironment for hematopoietic cell differentiation. The equilibrium of the human body and quality of life can be altered due to the changes on bone structure caused by injury or diseases (Eslaminejad and Bagheri, 2009; Salgado et al., 2004; Genant et al., 2010; Bose et al., 2012; Rajesh et al., 2012a).

## CURRENT MEDICAL TREATMENTS FOR BONE DEFECTS

In the current medical treatment, a variety of therapeutic modalities have been used to treat the healing of bone defects. The most widely used method is traditional bone graft technique which includes autograft, allograft, and xenografts. Other than that, metal and ceramic implants are also used to treat bone defects. All the treatment methods have advantages and disadvantages.

Autologous transplant technique involves the replacement of bone defects using patient's bone taken from another part. It is still considered to be a gold standard method for bone substitution among the bone graft techniques because it provides osteogenic, osteoinductive, and osteoconductive components to the defective site, which are needed for bone healing and regeneration. Even though, it provides good percentage of success, it has some limitation due to limited quantity of bone marrow, and it requires an extensive operation, which may cause morbidity, pain and infection to the donor site (Eslaminejad and Bagheri, 2009; Salgado et al., 2004). Allograft is considered to be a suitable alternative for autograft, which can overcome the problem of quantity for large bone defects as it could be derived from humans as well. However, the rate of graft incorporation is lower when compared with autograft. Bone for allograft surgery can be collected from either living or nonliving donors. Bone from donor site is osteoconductive but less osteoinductive, and often requires sterilization (gamma irradiation). Hence, there is a possibility of potential health risk of transmission of pathogen from host to donor, which eventually leads to infection in the recipient's body after the transplantation. HIV, HBV and HCV are some of the common pathogens transmitted. In addition to that, one fatal infection by *Clostridium Difficile*, and 26 bacterial infections have also been found (Salgado et al., 2004; Campana et al., 2014). A bone that is harvested from animal body can be used to reconstruct the defected area in xenograft techniques. From a theoretical point of view, xenografts possess significant disadvantages when compared with auto and allografts. It has the higher resorption rate and may exaggerate the inflammatory response, which may cause harmful effect for bone healing. There is greater possibility of transmission of several zoonotic diseases including bovine spongiform encephalitis, rabies and Epstein-Barr virus from animals to humans during the reconstruction of defected area through xenografts (Oryan et al., 2013; Campana et al., 2014).

The use of metal-based biostructural materials (stainless steel, titanium alloys and cobalt alloys) for load-bearing implants and internal fixation devices, such as orthopedic, dental implants and even vascular/nonvascular stents is the most common method due to the excellent mechanical strength and resilience of the materials. Generally, refractory metals including cobalt-chromium alloys, tantalum (Ta), niobium (Nb), and titanium (Ti) have also been used for implant studies due to their excellent corrosion resistance. The lower bioactivity of metallic materials can create a poor interface between the implants and biological host tissue. Metals do not degrade after implantation; hence, it requires secondary surgery to remove them from host. Moreover, they may release toxic ions, which trigger the immune response and infection in the host and leads to failure (Abtahi et al., 2012; Eslaminejad and Bagheri, 2009; Matsuno et al., 2001; Ren and Yang, 2013; Jung et al., 2015). Ceramic materials are used in dental restorations and arthroplasty applications can function as replacement for metallic substrate. These materials play a potential role in biomedical fields due to their biocompatibility, strength, chemical resistance and esthetic properties. The most commonly used ceramic bone substitutes are calcium-based substitutes including HAP and tricalcium phosphate (TCP). Other than this, different ceramic composites including alumina, zirconia, and yttria-doped tetragonal zirconia polycrystal (Y-TZP) based ceramic materials are also used for implants (Fig. 19.1). The major disadvantages faced by



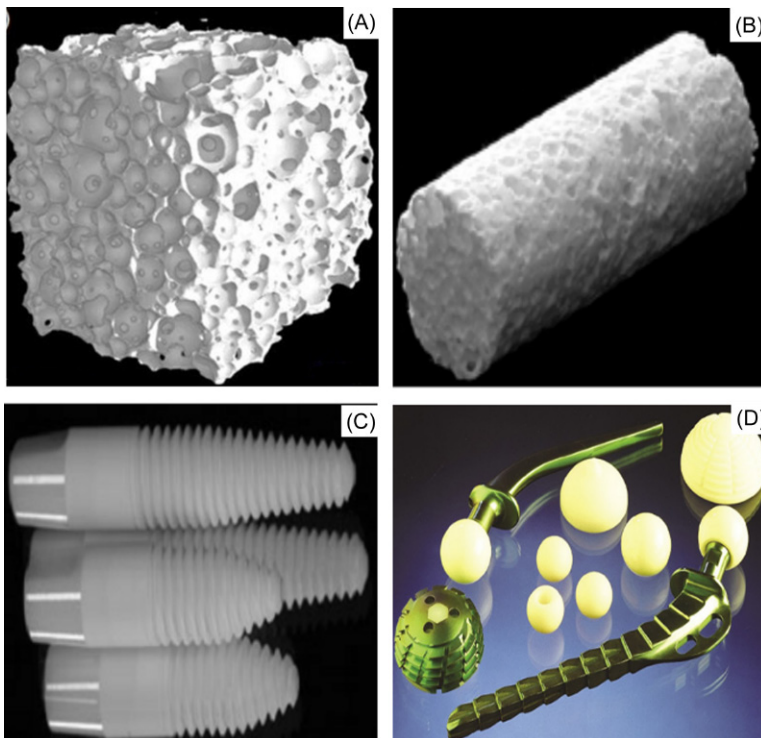
**FIGURE 19.1**

(A) HAP, (B) TCP, (C) Zirconia, and (D) alumina ceramic implants used for clinical study.

ceramic implants are its low tensile strength and brittleness. Thus, they cannot be used in locations of significant torsion, bending or shear stress (Salgado et al., 2004; Cavalu et al., 2014; Osman et al., 2014). Hence, it is clearly evident that an adequate bone replacement with full recovery of patients has to be found.

## BONE TISSUE ENGINEERING

Bone tissue engineering is a multifarious and dynamic process, which initiates begins with the migration and recruitment of osteoprogenitor cells followed by their proliferation, differentiation, matrix formation along with remodeling of the bone (Bose et al., 2012). Bone tissue engineering offers a promising approach for bone repair, and may potentially provide alternative solution than to those used currently. It has become evolved as rapidly an expanding research, and renders solutions to the problem associated with traditional autograft and allograft procedure, such as donor scarcity, supply limitation, and pathogen transfer and immune rejection (Fig. 19.2). Scaffolding material is introduced to either induce the formation of bone from surrounding tissue or to act as



**FIGURE 19.2** Basic principle and process involved in the tissue engineering.

a carrier or template for implanted bone cells or other agents. This method decreases the vascular insult of the implant to the bone, and causes less-stress shielding by minimizing the incidence of implant related osteopenia and subsequent refracture. The bone under this treatment typically uses an artificial ECM, osteoblasts and exhibits regulating factors that enhance cell population, growth differentiation and mineralized bone tissue formation. Scaffold is introduced to either stimulate the formation of bone from surrounding tissue or to act as a carrier for implanted bone cells (Burg et al., 2000; Liu and Ma, 2004).

BTE materials require the following criteria to be an effective scaffold:

1. Biocompatibility is one of the important requirements for bone tissue engineering scaffold, which has been described as its ability to support normal cellular activity of molecular signaling systems without any local and systematic toxic effects to the host tissue (Bose et al., 2012; Mourino and Boccaccini 2010).
2. The internal architecture of defected bone is strongly influenced by the strength of the scaffolds, and must be designed carefully. The mechanical strength possessed by an ideal scaffold should match the host bone properties and meet the proper load transfer requirements (Bose et al., 2012; Woodard et al., 2007).
3. Natural bone is porous and the porosity is filled with osseous medulla. The porosity, pore interconnectivity, and suitable flow channels must be present in scaffold for adequate cell invasion, transport of nutrients and waste, and transduction of cellular signals (Bose et al., 2012; Murphy et al., 2010; Pok et al., 2014).
4. Bioresorbability is considered to be a crucial factor for ideal scaffolds. The scaffold should not merely have the similar mechanical properties as that of the host bone but it should also have the ability to degrade with time. The degradation should be at controlled resorption rate until completion of growth of new bone tissues (Bose et al., 2012; Hutmacher 2000).

## POLYSACCHARIDES

A large number of components present in ECM containing adhesive molecules, notch-signaling molecules, traction-enabling adhesion molecules and proteoglycan molecules play a major role in binding and modulating the activity of a number of growth factors in tissue engineering. Secretion of growth factor initiates from the signal transmission mechanism and guides cell behaviour through binding with specific trans-membrane receptors in the target cells. The ECM brings cells together and controls the tissue structure, regulates the function of cells and allows the diffusion of nutrients, metabolites, and facilitates the tissue development. Therefore, it is necessary to explore biocompatible

materials, which have the ability to mimic the natural ECM and allow tissue regeneration (Lee et al., 2011; Mano et al., 2007).

Polysaccharides composed of monosaccharides linked together through glycosidic bonds as linear or branched. It can be divided into two natural polysaccharides and semisynthetic polysaccharide based on their sources. Natural polysaccharides derived from living organisms, such as algae, plants, microorganisms, and animals where as semisynthetic polysaccharides are produced by the chemical or enzymatic modification of the parent macromolecules. The various physico-chemical characteristic exhibited by polysaccharides are gelation, solubility, low osmotic effect, and surface properties depending on their composition and architecture. Polysaccharides and their derivatives take part in signal recognition and cell–cell communication. Further, they involve prominent roles in the immune system, fertilization, pathogenesis prevention, blood clotting, and system development. More importantly, it helps the formation of blood vessels in the surrounding tissue. Moreover, it can avoid the stimulation of chronic inflammation or immunological reaction and toxicity, often detected with synthetic polymer (Grandfield et al., 2009; Tonelli et al., 2012; Zong et al., 2012; Bacakova et al., 2014).

## ALGINATE

Alginate is a well diversified polysaccharide in the biomedical field because of its utility in preparing hydrogels at mild pH and temperature conditions, suitable for sensitive biomolecules like proteins and nucleic acids, and even for living cells, such as islets of Langerhans (Pawar and Edgar, 2012). Several alginate derivatives possessing various structures, properties and functions can be easily obtained via chemical and physical reactions. Further, alginate is readily processable as applicable three-dimensional (3D) scaffolding materials including hydrogels, microspheres, microcapsules, sponges, foams, and fibers. More importantly, the sufficient adhesiveness and hemostatic property towards osteoblast cells and as the supporting matrix for tissue repair make it more applicable towards tissue engineering (Sun and Tan, 2013; Venkatesan et al., 2015; Wang et al., 2009).

### Alginate Hydrogel

Hydrogel is an effective approach to deliver the cells for tissue engineering, which helps to transplant the cell and polymer combination in a minimally invasive manner. It possesses structural similarity to the macromolecular-based components in the body. Gellation through ionic or covalent cross-linking is considered to be a crucial factor in hydrogel preparation. As discussed earlier, alginate undergoes crosslinking with divalent anions (Lee and Mooney, 2001). But, ionic crosslinked alginate degrades in uncontrollable

and unpredictable manner via loss of divalent ions into the surrounding medium. Covalent crosslinking of alginate with other crosslinking densities has been tried to circumvent this problem through increasing the mechanical properties (Lee et al., 2000). Covalent crosslinking of alginate with poly(ethylene glycol; PEG) diamine showed controlled mechanical properties based on molecular weights of PEG (Eiselt et al., 1999). Likewise, covalent crosslinking with adipic dihydrazide, lysine, and poly(ethylene glycol)-diamines controlled the mechanical properties of alginate hydrogels along with hydrophilic/hydrophobic balance (Lee et al., 2000). Photo crosslinking is an alternate method and gained increasing attention recently. It is used to deliver the aqueous macromolecular solution containing cells and or bioactive components in a minimally invasive manner and undergoes rapid crosslinking upon exposure to ultraviolet (UV) light followed by photoinitiator to initiate the radical polymerization. Methacrylated alginates were photo crosslinked using UV in presence of 0.5% photoinitiator. It showed tunable mechanical and degradation rates by varying the degree of alginate methacrylation and exhibited low cytotoxicity when cultured with primary bovine chondrocytes (Jeon et al., 2009). Chou et al. (2009) prepared photo crosslinked alginate hydrogel to improve the inferior proteoglycan accumulation in vitro exhibited by ionic crosslinking. The hydrogels were implanted for up to 8 weeks to examine nucleus pulposus tissue formation for intervertebral disc regeneration, which increased assembly of type II collagen and proteoglycans (Chou et al., 2009).

Alginate gel crosslinked with calcium ions can be utilized in cartilage tissue engineering. The calcium crosslinked alginate hydrogels were showed increased differentiation and proliferation of the rat bone marrow cells depending on calcium composition (Wang et al., 2003). Covalent crosslinking of alginate hydrogel with RGD peptide promotes the cell adhesion behavior (Rowley et al., 1999). Alsberg et al. (2001) prepared RGD peptide modified alginate hydrogel which promoted osteoblast adhesion and spreading of MC3T3-E1 cells with increased cell differentiation. Further, transplantation primary rat calvarial osteoblasts revealed improved in vivo bone formation with G4RGDY-modified alginate compared with unmodified alginate (Alsberg et al., 2001). As an interesting approach, shear-reversible crosslinked alginate hydrogels have been tried to recover cell structures from shear induced breakdown. Park et al. (2009) has reported shear-reversible alginate hydrogels by combining crosslinking of cell-crosslinking and ionic crosslinking techniques. The small addition quantity of calcium ions decreased the number of cells required to form cell cross-linked hydrogels without changing the shear reversibility. And, the concentration of both the cells and calcium ions play an important role in physical properties and gelation behavior of the prepared gels. In vivo study revealed that alginate gel prepared by combination method was useful in cartilage tissue engineering (Park et al., 2009).

## Alginate-Polymer

Alginate has shown poor cell attachment *in vitro* due to its hydrophilicity and poor osteoconductivity. Composites prepared using combination of alginate with other polymers including chitosan, collagen, gelatin and hyaluronate have improved the cell adhesion behavior of alginate towards bone tissue engineering ([Bendtse and Wei, 2005](#)). Following studies support the improved properties of composites.

- (1) Chitosan, a naturally occurring cationic polysaccharide; promotes cell adhesion, proliferation, and differentiation, and evokes a minimal foreign body reaction on implantation ([Hutmacher et al., 2001](#); [Suh and Matthew, 2000](#)). It is osteoconductive and enhances bone formation ([Muzzarelli et al., 1994](#)). The Li group reported chitosan-alginate scaffold with improved mechanical and biological property compared with alginate alone. Bone forming osteoblast cells readily attached to the chitosan-alginate scaffold and proliferated well ([Li et al., 2005](#)).
- (2) Collagen, a major component of ECM presents in natural bone ([Baniasadi and Minary-Jolandan, 2015](#); [Guan et al., 2015](#)). [van Susante et al. \(1995\)](#) studied the effect of collagen and alginate as carrier for chondrocyte. Collagen showed increased number of cell proliferation but slower dedifferentiation of chondrocytes where as alginate showed initial loss of cell but maintained chondrocyte phenotype. The high proliferation rate of chondrocytes in collagen and maintenance of chondrocyte phenotype in alginate may provide efficient bone cell formation when combined to prepare composite ([van Susante et al., 1995](#)).
- (3) [Balakrishna et al. \(2014\)](#) reported self cross-linking periodate oxidized alginate and gelatin scaffold in presence of borax. Cell adhesive gelatin improved the chondrocyte cell proliferation of scaffold and can be used for the treatment of osteoarthritis ([Balakrishna et al., 2014](#)).

## Alginate-Calcium Phosphate

Bioactive calcium phosphate ceramic including HAP,  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) are widely used in surgery to mimic and replace the broken bone (bony defects in orthopedic, maxillofacial surgeries, and dentistry) because of its biocompatibility, bioactivity, osteoconductivity, osteoinductivity and a structure resembling the inorganic component present in the bone ([Ivankovic et al., 2012](#); [White et al., 2007](#)). More importantly, HAP has been identified as a well-known piezoelectric material. Electrical polarization is also considered to be an important criterion for bone mineralization in living animals. The polarization in HAP has been found to simulate the bone like apatite both

in vitro and in vivo (Lang et al., 2013). Reinforcing osteoconductive calcium phosphate ceramic with alginate improves the biological and mechanical limits of alginate for BTE (Turco et al., 2009). The addition of 0.32% of HAP to the alginate hydrogel increased the dynamic stiffness in compression as high as 400% (Bouropoulos et al., 2009). Further, alginate-HAP scaffold prepared by phase separation method at  $-40^{\circ}\text{C}$  with a 50% HAP showed best mechanical properties. Furthermore, UMR106 cells seeded on to the scaffolds showed better cell attachment to the 75/25 and 50/50 alginate/HAP when compared with pure alginate scaffold (Lin and Yeh, 2004).

Sangeetha et al., found that addition of gelatin on in situ method preparation of alginate/HAP composite influences the crystallinity of apatite formation along with microstructure of the resulting composite (Sangeetha et al., 2013). Human bone marrow stromal cells (hBMSCs) were cultured on alginate-gelatin-HAP scaffold for 3 weeks. In vitro analysis revealed that 10–14-fold increase of cell number after 2 weeks of cultivation and also increased alkaline phosphatase (ALP) activity for osteogenic-induced hBMSCs (Bernhardt et al., 2009). The addition of alginate into the HAP/collagen composite expands its limited applications in BTE. Active bone formation was observed around the HAP/collagen-alginate hybrid material along with tissue invasion when compared with simple alginate and HAP. HAP/collagen-alginate efficiently carried the recombinant human bone morphogenetic protein 2 (rh-BMP2) and showed bone formation over a period of 5 weeks after implantation (Sotome et al., 2004). Jin et al. (2008) synthesized porous HAP/chitosan-alginate scaffold containing different amounts (0%, 10%, and 30%) of HAP by in situ coprecipitation method. The pore diameter decreased with increasing HAP content to 30% but the compressive strength increased with increasing HAP (Jin et al., 2008). HAP/chitosan-alginate scaffold also used as rh-BMP2 carrier, which differentiates into bone marrow mesenchymal stem cells (MSCs) (He et al., 2014). Lists of calcium phosphate ceramic reinforced alginate composites prepared for BTE were shown in Table 19.1.

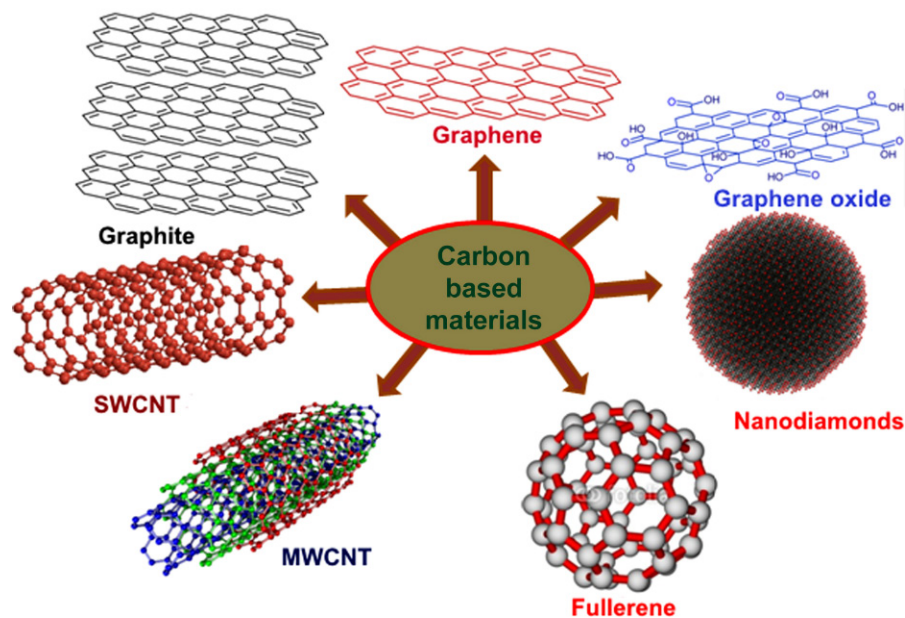
**Table 19.1** Lists of Calcium Phosphate Ceramic Reinforced Alginate Composites for BTE

S. No.	Composite	References
1.	Alginate-HAP	Parhi et al. (2006); Brun et al. (2011)
2.	Alginate-chitosan/HAP	Han et al. (2010); Jin et al. (2012)
3.	Alginate-gelatin/HAP	Teng et al. (2006); Bernhardt et al. (2009)
4.	HAP/collagen-alginate	Sotome et al. (2004)
5.	$\beta$ -TCP-alginate	Matsuno et al. (2008)
6.	Calcium titanium phosphate-alginate	Ribeiro et al. (2004)
7.	Chitosan-gelatin/ $\beta$ -TCP	Yin et al. (2003)

## Alginate-Carbon-Based Nanomaterial

Carbon-based nanomaterials have gained the attention of scientific community and have been extensively used for several applications due to their unique properties, such as light weight, high surface to volume ratio, mechanical strength, thermal stability, optical, electronic, magnetic and biological properties. The surface property of the carbon-based nanomaterials improves their interaction with many biological entities. Such interactions depend upon several factors including fabrication method, size and specific geometry of nanomaterials. Hence, interactions with biological entities and multiple functionality of carbon-based nanomaterials gained attraction towards biomedical research. The stability and selectivity of nanomaterials depend on their properties, such as hydrogen bonds,  $\pi$ - $\pi$  stacking, dispersion forces, dative bonds, and hydrophobic interactions. A wide range of carbon-based nanomaterials are available (Fig. 19.3) including carbon nanotube (CNT), graphite, graphene (GP), graphene oxide (GO), nanodiamonds, and fullerenes (Cha et al., 2013; Ravichandran et al., 2015; Scida et al., 2011; Zhang et al., 2013). Among them, CNT and GP based materials are most widely used in biomedical applications.

CNT and GP based alginate composite with improved mechanical strength have not been explored fully. SWCNT-alginate hydrogel resulted faster gelling and higher mechanical strength than the alginate hydrogel (Kawaguchi



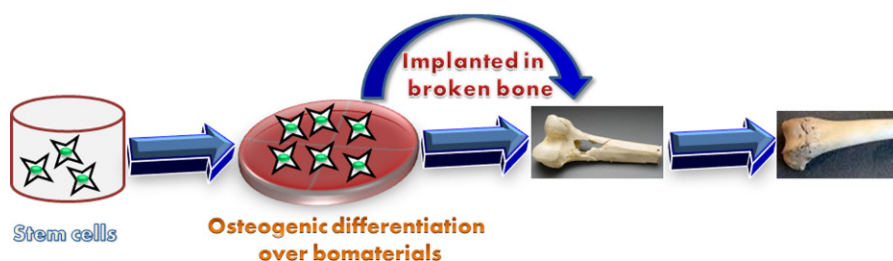
**FIGURE 19.3** Images for some of the carbon-based nanomaterials. SWCNT-single walled carbon nanotube, MWCNT-Multiwalled carbon nanotube

et al., 2006). Islam et al. (2012) prepared CNT-alginate scaffold by electro spinning method, which showed improvement in tensile strength. Transmission electron microscope (TEM) study on bovine serum albumin (BSA) immobilized in double walled carbon nanotube (DWCNT) doped alginate gel proved adsorption of BSA on DWCNTs and the uniform distribution of DWCNTs in alginate. DWCNT-alginate can be used as biocomposite in BTE (Ma and Wen, 2008). Yildirim et al. (2008) applied freeform fabrication technique to prepare 3D SWCNT-alginate scaffold. The structure of the SWCNT-alginate scaffold altered by the addition of SWCNT and in vitro study by rat heart endothelial cell showed improved cell adhesion and proliferation (Yildirim et al., 2008). Tricomponent composite scaffold of oxidized MWCNT-alginate-HAP was prepared by freeze drying method with interconnected pore structure. The pore size and porosity were found to be 130–170  $\mu\text{m}$  and 93.85% respectively which meets the requirement for BTE. Further, the in vitro analysis on MG 63 cell line showed better cell proliferation and ALP activity (Rajesh and Ravichandran, 2015).

GP based alginate composite was prepared by different methods and each methods showed different mechanical strength. He et al. (2012) prepared alginate/GO fibers using wet spinning method. The tensile strength of alginate/GO increased from 0.32 to 0.62 GPa and Young's modulus increased from 1.9 to 4.3 GPa at 4 wt.% GO loading (He et al., 2012). Inoita et al. (2013) synthesized alginate/GO composite film by casting of a suspension of two components. The tensile strength increased from 71 MPa to 0.85 GPa and Young's modulus increased from 113 MPa to 4.18 GPa for the alginate films containing 6 wt.% of GO. It was observed that strong hydrogen bonding and high interfacial adhesion between GO and alginate improved the thermal and mechanical properties of composite (Inoita et al., 2013). In GO/carboxymethyl cellulose/alginate composite blends prepared by a solution mixing and evaporation method, the incorporation of 1 wt.% of GO to the carboxymethyl cellulose/alginate composite blend improved the tensile strength and Young's modulus by 40% and 1128% respectively (Yadav et al., 2014). However, the compressive strength of GO-alginate-HAP composite scaffold prepared by freeze drying method was found to 171 MPa. Further, GO-alginate-HAP scaffold improved their osteoconductivity on MG 63 cell lines. Hence, GO based alginate composite can play a promising role in BTE (Rajesh and Ravichandran, 2015).

### Alginate-Stem Cells

Stem cells including adipose tissue-derived stem cells (ADSCs), bone marrow-derived mesenchymal stem cells (BM-MSCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), muscle-derived stem cells (MDSCs) and dental pulp stem cells (DPSCs) have gained wider attention in bone tissue



**FIGURE 19.4** Schematic representation for the biomaterials guided differentiation of stem cells to regenerate broken bones.

engineering (Fig. 19.4). These stem cells have the capacity to differentiate into osteogenic lineage but require biomaterials to induce in vitro differentiation into bone forming cells, osteoblasts (Seong et al., 2010). Alginate material can provide microenvironment, and possesses the hydration ability with good biocompatibility (Zhou and Xu, 2011). The chondrogenic differentiation of ADSCs seeded alginate scaffolds increased the rates of protein, proteoglycan, sulfated glycosaminoglycans, and hydroxyproline. Moreover, Chondrogenic culture conditions did not affect the mechanical property of alginate hydrogel over 28 days of culture and support its differentiation (Awad et al., 2004). Polylactic acid-alginate scaffolds were seeded with BM-MSCs and differentiated in vitro with transforming growth factor  $\beta$ . The implantation of the constructs in osteochondral defects filled with a cartilaginous tissue after 6 weeks (Wayne et al., 2005). MSCs and BMP-2 were combined to prepare injectable chitosan-alginate gel/MSCs/BMP-2 composite for new bone formation. The composites were injected into the subcutaneous space on the dorsum of nude mice and the histological examination over a period of 12-week confirmed the new bone formation (Park et al., 2005). As a new approach, anti-BMP2 monoclonal antibodies were coencapsulated with MSCs in alginate microsphere to induce BMP for osteo-differentiation of progenitor cells. This codelivery system provides microenvironment for stem cells with captured BMP signals and will be a promising treatment modality for regeneration of craniofacial, axial and appendicular bone defects (Moshaverinia et al., 2013).

Alginate microbeads, oxidized alginate microbeads and oxidized alginate-fibrin microbeads with sizes of about 100–500  $\mu\text{m}$  were fabricated with MSCs. Alginate microbeads undergo slower microbead degradation with very few cell released at 21 days whereas oxidized alginate microbeads started to degrade after 14 days. At 21 days, the oxidized alginate-fibrin microbeads possess 4 fold and 15 fold increased live cell density over oxidized alginate microbeads and alginate microbeads respectively. Hence, oxidation and addition of fibrin to the alginate plays a key role in osteodifferentiation, and bone mineral synthesis (Zhou and Xu, 2011). ESCs derived MSCs seeded alginate microbeads encapsulated

macroporous calcium phosphate cement had excellent cell viability and ALP expression from 1 to 21 days and is a promising construct for bone regeneration in a wide range of orthopedic and maxillofacial applications (Tang et al., 2012).

## CONCLUSIONS

Alginate is a well known polysaccharide utilized for BTE next to chitin and chitosan because of its low cost, biocompatibility, biodegradability, rapid gelation pore forming ability and encapsulation efficacy. The poor cell adhesion behavior and mechanical strength are the limitations to use for hard tissue engineering. Materials like chitosan, collagen, gelatin and RGD peptide included alginate showed improve cell adhesion behavior for better proliferation. Bioactive ceramics (HAP,  $\beta$ -TCP) and carbon-based nanomaterials incorporated alginate improved the mechanical properties along with biological activity to meet the requirements for natural bone. Further, the inclusion of stem cells to the alginate composites has improved the regeneration of broken bone.

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## Further Reading

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