Chapter

Bone Mineralisation

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Abstract

The mineralisation term mentions the development of inorganic precipitation over an organic background. This process occurs in a life span of biological organism for the formation of bone, teeth, exoskeletons, egg shells, etc. So, basically bone mineralisation is defined as the process of deposition of minerals on the bone matrix for the development of bone. The human bone is made up of 60–70% minerals which include calcium phosphate in the form of hydroxyapatite followed by 20–40% organic matrix containing type I collagen fibres and less than 5% of water and lipids. During bone mineralisation process osteoblasts which are also known as bone forming cells, aids to the production of calcium phosphate crystals which are then aligned in the collagen based fibrous matrix. The bone mineralisation procedure also known as calcification is a lifelong activity of a human being.

Keywords: bone, mineralisation, calcium phosphates and collagen fibres

1. Introduction

Bone is a multifaceted system which behaves as mechanical shield for providing support and security. Bone also plays an important role in haemostasis. Recently, it came to observation that bone also aids in functioning of endocrine glands. To fulfil all these purposes, bone is a well architecture behaving as a functionally graded structure from millimetre to nanometre range. As a result of this gradation bone shows an unusual amalgamation of high stiffness and toughness which are frequently inversely associated. Broadly, bone is made up of organic and mineral part where the organic part is comprised of type I collagen whereas the mineral part contains the calcium deficient hydroxyapatite. The mineral part of the bone is interlinked with collagen in such an organised manner that it not only provides flexibility and ductility to the structure but also shows stiffness. This organisation of bone when observed at nanoscale range it behaves as a composite which acts as a shield to the brittle hydroxyapatite from damaging and also helps in carrying load by transferring forces around the bond hence thereby reducing the stress formation in the composite matrix. The fibres of the collagen intermingled with mineral part are arranged in very different manner when observed on microscopic range. This arrangement at micrometre level completely depends on the rate of bone formation or bone location on the substrate. This is due to the various functions played by the bone tissue, i.e., during fracture rapid bone formation development of bone during growth, unhurried bone formation in order to adjust in accordance to mechanical requirements so as to avoid any damage to its structure as well as maintaining its mechanical assets. Bone can be divided into two major categories depending on its mechanical and biological aspects i.e. cortical bone (compact/dense) and cancellous (porous/spongy bone namely trabecular) [1].

2. Bone composition

The mineral portion of the bone which basically comprises of hydroxyapatite contributes to the 65% of weight of bone. The remaining 20–30% of the bone weight composed of collagen of Type I. And, the last 10% of weight includes water molecules which are present collagen-mineral structure. Also, there are some free water molecules in the bone composite which gets redistributed during load bearing phase of bone. These free molecules pass through the vascular channels of bones and hence play a pivotal role in detection of signals by cells thus transferring information regarding load bearing environment. The interaction between bone mineral and unbound water molecules in a ratio of 1:1 helps in the process of bone mineralisation i.e. when the amount of water declines the mineralisation of bone starts to proceed and vice versa. The bone mineralisation is directly related to the stiffness of the bone as bone tends to grow stiffer since it contains higher amounts of mineral and lesser quantity of water. And as a result of this stiffened bone becomes more prone to brittleness and hence fracture easily. Approximately, 90% of organic part of bone is made up of collagen of type I category also collagen of type III and V are present in very minor quantities. And, the last 10% belong to non-collagenous proteins which help in the regulation of collagen development and management of fibre size, resistance to micro-crack, cellular adhesion and mineralisation. Around 85% of non-collagenous proteins are present in extracellular matrix and rest resides with the bone cells.

2.1 Collagen

The fibres of collagen are intertwined with the plate like structure of bone. Each and every collagen molecule exists as a triple helical structure formed from two chains of α 1 and one chain of α 2. Individual chain has around 1000 amino acids lengthwise and the centre part of the helix contains triplets of gly-X-Y in repeating sequence. The glycine molecules help in the formation of helical structure of collagen. Basically, all the amino acids are incorporated in collagen but the X and Y units are often composed of residue of hydroproline and proline. The maintenance of helical rigidity of the chain is due to the proline and hydroproline residues. The hydroxyl part of the hydroproline amino acid is important for interaction with water molecules via hydrogen bonding. The triple helix structure of collagen retained by the water molecules is affined toward hydroxyproline. At intercellular stage, peptides of non-helical region, i.e., N-propeptide as well as C-propeptides hold the chains at one place by cross- linking with sulphur. The propeptides present at the terminal point of triple helix are called procollagen molecule. During the exocytosis of molecules, the propeptide parts are broken down enzymatically resulting in non-helical parts at the molecular end N or C-terminal respectively. The enzymatically cleaved peptides result in the development of mature collagen molecule which has a pattern of non-helical N- and C-terminal peptides and helical nature of triple helix region. The microfibrils of collagen are semi-hexagonal system of five collagen molecules. The lateral and longitudinal combination of microfibrils leads to the formation of fibres approximately 10 μm in length and around 150 nm in diameter. The collagen fibres when observed under electron microscope showed a band pattern of around 67 nm. This band pattern is known as D-banding and it demonstrates the area between the neighbouring ends of collagen molecules as well as the overlapping between the lateral neighbouring molecules present at the end sections. In an osteoporotic bone the average diameter between the collagen fibrils and their spacing is very less as compared to a normal bone. The collagen fibrils are joined with the help of various types of cross-links which may have influential

effects on the materialistic properties of the surrounding tissues and thereby affecting the mechanical traits of the whole bone system. The crosslinks bonds which connects the collagen fibrils are broadly categorised into enzymatic crosslinks and non-enzymatic crosslinks which forms a AGEs (advanced glycation end products).

2.2 Non collagenous proteins

There are various proteins of non-collagenous nature that helps in the regulation of formation and preservation of the extracellular matrix. Even though they comprise only of 2% of bone in weight but NCPs do a very important job during embryogenesis and formation as well as establishment of fibrils of collagen. It also regulates mineral of formation of bone and deliver channels for all signalling regarding the attachment of cells. The NCPs are divided into

1. *Proteoglycans*: They are basically consisting of various heparin sulphate, hyaluronan, small leucine rich proteoglycans called SLRPs. These are wide range of molecules where the main proteins are covalently bonded to the lateral chains of sulphated glycosaminoglycan. The proteoglycans which are present in the bone are smaller in size as compared to the other non-bone region proteoglycans. It manages the nucleation of apatite and the growth of apatite which in turn controls mineralisation.

2. *Glycoproteins*: It consists of fibronectin, thrombospondin (TSP1 and TSP2), vitronectin and lastly, alkaline phosphatase (ALP). In bones there are huge numbers of glycoproteins, out of which functions of few glycoproteins are not known. The activity of ALP helps in determining mineralisation factors because it undergoes hydrolysis with pyrophosphates which, as a result restricts the deposition of minerals by tagging itself with mineral crystals. If the pyrophosphates are neutralised, then this leads to normal growth of mineral crystals and as a result regulates the bone mineralisation. Since ALP is solely not produced by bone but also by kidney and liver any changes in the levels of ALP will not give any precise results regarding the mineralisation activity. Nonetheless, if the ALP is taken from the bone specific region, then the levels of ALP may determine the activity of bone mineralisation. In fact, lower levels of ALP or dysfunctionality of ALP leads to disorder recognised as hypophosphatemia which results in hypercalcemia causing death in children. TSP1 (Thrombospondin1) and TSP2 (Thrombospondin2) are found in mesenchymal stem cells and chondrocytes in the process of development of cartilage in the primary steps of bone development. TSP2 enhances the process of mineralisation as well as it escalates the osteoid process during mineralisation. Both vitronectin and fibronectin attaches to the cells where the vitronectin helps in attachment of cells which are found in plasma membrane of osteoclast and works with the osteopontin for binding osteoclasts to the matrix of mineral. The fibronectin plays an important role in cell proliferation during formation of bone.

3. *SIBLING* also known as small integrin binding ligand N linked glycoprotein which includes dentin matrix acidic phosphoprotein 1 (DMP1), osteopontin, sialoproteins and MEPE (matrix extracellular phosphoglycoprotein). In the preliminary stages of osteogenesis osteopontin is secreted. The ostoponin exists near the periodontal region of teeth as well as the cement line of bone. It restricts the crystal growth during mineralisation, also it attaches itself to the osteoclast in order to enhance the binding of osteoclast to the mineral surface of bone in the course of bone resorption. DEMP1 has an immense inclination

for hydroxyl apatite and N-telopeptide region of type I collagen, also is indicated by osteocytes and osteoblasts. It helps in local regulation of bone mineralisation. Although, it is quite an unknown fact whether DMP1 is involved in the differentiation process of osteoblasts to osteocytes. But in adequate DMP1 results in hypophosphatemia rickets due to increased level of FGF23. MEPE also belongs to SIBLING genre which functions to locally regulate Mineralisation. It is mainly found in osteocytes and odontoblasts, where it is supremely demonstrated in tissues which are still under Mineralisation such as modification of intramembrane and endochondral plaited bone of your fractured callus. *In vivo* results show that lack of MEPE gives increased bone mass as well as decreased in bone loss.

4. *Osteocalcin (Gla bone protein)*: The osteocalcin is indicated in osteoblasts as well as osteocytes. It helps in binding o calcium and deposition of mineral. As a result, it is considered as an indicator of bone formation. However sometimes It may also behave as a precursor in regulation of osteoclasts. It has been seen that mice in absentia of osteocalcin suffers from grave osteoporosis. Hence, it can be seen why osteocalcin is considered as one of the important marker for bone remodelling. It has been also observed that post-menopausal osteoporosis that its increased level increases bone remodelling rate causing acute imbalance between resorption and formation of bone.

5. *Osteonectin* are also known as SPARC, i.e., secreted proteins acidic rich in cysteine. It is present at mineral deposited location where it bonds with collagen, hydroxyapatite and vitronectin. It helps proliferation of freshly nucleated mineral crystals. Since its plays a vital part in osteoblasts growth, its non-existence causes osteopenia i.e. low bone density. It bandages itself to various growth factors such as FEF2, PDGF (platelet derived growth factor), VEGF (vascular endothelial growth factor) for the regulation of mineralisation.

3. Bone mineral

Bone mineral is made up of carbonate apatite which has poor crystalline structure. The apatite undergoes nucleation in the space between collagen fibrils end called hole zones and it spreads longitudinal over the collagen fibrils. In the beginning, mineral is settled in the form of amorphous calcium phosphate followed by calcium carbonate in good amount. The carbonate proportion reduces during bone tissue maturation, also mineral crystals form disc like structure growing laterally while aligning themselves parallel to the fibrils of collagen. The l-axis (long axis) of mineral disc oriented with the longitudinal line of bone. The typical size of mineral crystals has less than 10 nm thickness. Gradually, the mineral disc merge with other crystals to form a large polycrystalline structure, which becomes indeed larger than the thickness of fibrils. The mineral crystals grow more in size during bone ageing due to changes in ion contents of mineral composition. The age of the tissue is directly proportional to the size of the crystals. Although, it is tough to differentiate amid small crystals bearing numerous defects and large crystals having less defects, as both shows likewise crystalline behaviour. The soluble carbonate which adheres to surface of crystals can also be filled in by hydroxyl and phosphates groups of carbonate apatite. As a result, it helps in easy resorption of mineral. During the incidents of acid load, bicarbonate (-HCO_3) is being absorbed so as to maintain the pH of blood. The deficiency of -HCO_3 is balanced by the presence of carbonate and phosphate ions in bone mineral. When there is an abnormal production of acid, the

bone mineral tank helps in the maintenance of acid–base balance, which also many times leads to loss in bone mass. There are certain cations such as Mg, Na, strontium in place of calcium ions and fluoride ions in place of hydroxyl ions in apatite matrix. Also, these kinds of substitutions can cause changes in mechanical properties of bone as well as in the behaviour of osteoclast and osteoblasts. Previously, for osteoporosis sodium fluoride used as an anabolic remedy. It was observed that sodium fluoride promoted pre-osteoblasts and osteoprogenitor cells hence stimulating uninterrupted formation of bone without the initialization of resorption. However, it has been seemed that carbonate apatite is less unaffected by resorption than fluoro-apatite. Also, replacement of fluoride ions into the mineral leads to escalated brittleness of bone thus causing the bone prone to fracture. The poor mechanical behaviour of bone is only due to the replacement of fluoride ions from bone as compared to the occurrence of substitutions of other ions from the bone mineral. The accumulation of bone mineralisation takes place in two consecutive phases. The first phase is the rapid nucleation of the primary mineral crystals. This phase is also known as primary Mineralisation. And, second phase relates to the slow proliferation and development of the primary crystals up to a size of 40 × 3 × 7.5 nm. In the course of primary mineralisation crystals are very quickly accumulated in the collagen network thus attaining 65–70% of total mineralisation within 3 weeks approximately. During the second step, the mineral is deposited at a steady rate but in a more efficient way, till the mineral attains the required bodily limit, which may vary from months to year [1–19].

4. Bone cells

Bone is a structurally and metabolically very complicated organ which is a composite of mineral, collagen material and bone cells [20]. The bone cells basically include osteoblasts, osteoclasts and osteocytes, which are found in mesenchymal stem cells known to accumulate osteiod before the mineralisation process takes off, thereby helping in bone formation [21, 22]. Osteoblasts are known for regulating mineralisation and in the formation of extracellular matrix. They are originated from bone and are in cuboidal form especially found at bone surface and carries out the function of resorption [21, 23]. The quantity and function of osteoclasts are dependent on many factors such as proliferation, differentiation, rate of resorption by already developed osteoclasts and lastly cell lineage allocation [24]. They are the derivative of multinucleated polarised cells which are migratory in nature with good source of lysozyme enzymes [25]. They consist of mitochondria of pleomorphic type, vacuoles and lysosomes [26]. The formation and resorption of bone is the joint activity of osteoclasts and osteoblasts. And the factors which are involved during this process are prostaglandin E2 (PGE2), transforming growth factor beta 1 (TGF-β1), fibroblast growth factor, parathyroid hormone (PTH), osteoprotegerin ligand OPGL also known as RANKL (receptor activator of nuclear factor kappa B [(NF-κB) ligand]) and TRANCE i.e. TNF related activation induced cytokine. Resorption can also effect the biomechanical activities of bone for instance, formation of strong bone from a weak one [24]. Majority of bone cells are in the nature of osteocytes, thus comprising of 90–95% in the skeleton of an adult. The mature osteoblasts in the bone matrix are recognised as osteocytes. And they help in responding to the mechanical strain thus generating signals which can further coordinate the bone resorption and its formation [27]. Osteocytes that are present in mature bone are joined together with long extensions of cytoplasm that form small capillary like structure called lacunae or canalcali for the transfer of nutrients and wastes. Osteocytes are spread across the mineral matrix and connect to the

surface of bone and bone marrow via dendrites which involve osteoclast precursors for the stimulation of bone resorption and regulation of differentiation of mesenchymal stem cells [28–32]. The roles which are played by osteocytes and lacunae/canalcali involves restriction of fatigue cracks, exchange of mineral, hormonal stimulation for detection of stress or strain, mending of microdamage, modelling or remodelling of bone under mechanical criteria, osteocytic osteolysis, regulation of osteoclastic cutting cone during exchange of mineral and reformed remodelling act after the resorption [27].

During the bone mineralisation, the crystals of mineral are accumulated in a systematic manner over the extracellular matrix, where the cells surrounding the mineral matrix prepares a pattern for mineral accumulation thus commencing the location for mineralisation and fixing the final dimensions of mineral crystals. Although, various studies have been conducted throughout the world for determining the mechanism for formation of mineral crystals in every organism, but the exact explanation related to this mechanism remains unclear [33–35]. In accordance to the conventional theories about biomineralization, NCPs were actively engaged in the process of matrix mineralisation. In 1994, Hunter and Goldberg postulated that the effects of mineralisation for BSPs were completely connected to the glutamate and aspartate-rich sequences of peptides [36]. Later around 1997, Stubbs et al. carried out studies to consider the involvement of other groups such as sulphate, phosphate sialic acidic groups in the process of mineralisation [37]. According to earlier reports, nucleation of mineral takes place in their principal ionic solution which is supersaturated in nature. During nucleation in solid phase, a critical size of crystal is required in order to initiate the nucleation. This mechanism is known as stochastic solute clustering [38, 39].

At present, two important models have been considered for bone mineralisation process. The first model which involves mineralisation with the help of collagen template and the second model include the matrix vesicles for mineralisation purpose. It has been widely accepted that mineral formation is a systematic procedure which can never take place in the absence of matrix. Basically, the matrix gives an ordered pattern of deposition of mineral, thus directly participating in the mineralisation by behaving as a nuclear. It has been also seen that different mineralised tissues have different matrices [40]. Alternatively, matrix vesicles are the particles derived from extracellular matrix having a diameter of 100 nm, precisely positioned inside the bone matrix and the matrix of cartilage and peridentin. They provide the initial location for the calcification of all skeletal tissues. They are generally formed from a polarised bud which gets discharged from surface of the chondrocytes, osteoblasts and odontoblasts [41, 42]. The matrix vesicles are considered for preliminary location for mineral build-ups in bone tissue [43, 44]. In course of mineralisation involving cells, the formation of primary hydroxyapatite crystals takes place inside the vesicle membrane matrix [45].

a. *Collagen-moderated mineralisation*: In this type of mineralisation, the template for accumulation of mineral is provided by the collagen present in the bone tissue. And, these very collagen fibrils decide the sizes of crystals that can attained for the process of mineralisation. On the other hand, mineralisation does not take place in a deficiency of NCPs because they behave as molecules that generate signals all through the course of mineralisation [40, 46]. It was observed that BSPs role as a crystal nucleator, affected the osteocalcin recognition and remodelling of mineralised surfaces [36, 37, 47–51]. However, osteopontin and osteonectin helped in regulation of crystal formation on the basis of size, type and growth [52, 53]. During the growth phase, the crystals nucleates from an amorphous phase were the intervallic pattern of 67 nm cross-striated

collagen fibres [54, 55] carryover the nucleation in the 40 nm long gap sparsely dense zone. The aforementioned process was recommended to be guided by heavily acidic NCPs [56–59]. The basic principal of collagen based template mineralisation focuses mainly on the job of collagen fibrils during bone Mineralisation. When the mineralising fibre were observed under cryo-transmission electron microscope, it was found that polyaspartic acid which has soluble behaviour plays an integral part in collagen mineralisation [56, 60]. The calcium triphosphate ions complex is formed from the prenucleated clusters of acidic polypeptide [38]. These clusters are negatively charged; as a result, they get attracted towards the positive part on collagen [56]. Consequently, these ionic complex gets fused inside the collagen fibrils and then transform into a solid amorphous mass which further grows into an ordered apatite crystal complex regulated by the arrangement of collagen fibrils [40].

b. *Matrix-vesicle moderated mineralisation*: In this type of mechanism, the preliminary phase starts off at the mineral visceral where the Ca^{2+} ions and the inorganic phosphate (Pi) are formed *in vivo* [41, 61, 62]. The annexins and phosphatidylserine are the calcium binding molecules which tag them with BSPs so as to invite and regulate the deposition of calcium and phosphate ions previous to the creation of crystals of insoluble hydroxyapatite [62, 63]. During, this phase the pH of the intravesicules rises above due to the activity of carbonic anhydrase found in mineral vesicles [64], causing the stabilisation of primary mineral crystals [42]. In the second phase of matrix moderated mineralisation, the breakdown of mineral vesicles membranes takes place where the already formed hydroxyapatite are exposed to extracellular fluid [61]. The extracellular fluid comprises of matrix vesicles with homeostatically regulated levels of PO_4^{3-} and Ca2+ in order to help in proliferation of new hydroxyapatite crystals onto the already formed hydroxyapatite crystals. The perforations in the matrix vesicle membrane are carried out by proteases [65] and phospholipases [66]. The metalloproteinases of matrix vesicles which have the capability of degenerating mineral deficient proteoglycans helps in transferring of mineral towards itself [67]. In the recent studies, it has been observed that collagen type II and X bind to the outside surfaces of matrix vesicles thus acting as a channel for the transfer of crystals into the extravesicular matrix [68]. Three promising functions of matrix vesicles have been identified during the course of mineralisation. The first function involves the control of ion concentration by the matrix vesicles inside the matrix so as to start off the mineralisation around the collagen fibrils. Also, it controls the compositions of ions necessary for the formation of intravesicular apatite crystals thus starting the process of mineralisation with the transfer of ions to the collagen. And lastly, when the mineral vesicles interact with the collagen, the deposition of mineral onto the surface of fibrils is carried out [40].

5. Pathological mineralisation

Mineralisation is categorised into physiological or pathological types depending on the type of bone tissues i.e. hard bone tissue or soft bone tissue. Physiological mineralisation is required for the development of skeletal tissues in order to carry out daily functions of a normal human life. The second category of mineralisation is pathological mineralisation also known as ectopic which involves the mineralisation of soft bone tissues such as cartilages (articular cartilage) and tissues surrounding cardiac vessels that causes diseases and death. Recently, it has been found that the

reasons and aspects which cause physiological mineralisation can be similar to that of pathological. Lately, it has been reported both the mineralisation are instigated by the matrix vesicles where the particles which resides inside the membrane are released from the plasma membrane of mineralisation cells. The activators and regulators which cause pathological mineralisation are the same activators and regulators which will cause pathological mineralisation. It has been also reported that apoptosis causes physiological mineralisation and it has been seen that if physiological mineralisation happens to happen take place after injury of tissue, can prompt pathological mineralisation around the damaged or the injured tissue [69]. The mineralisation process is important for imparting mechanical properties in the bone [70]. As a result, when not regulated properly can lead to the inadequate or extreme mineralisation. Therefore, bone tissue quality gets jeopardised and becoming the cause for many bone related diseases. Osteomalacia is one such condition where the disease is caused in adults due to deficiency of bone mineral or excessive bone resorption. Rickets is the osteomalacia in children. As already explained, in a fit and mature bone, osteoclasts eliminate bone whereas osteoblasts accumulate osteoid and thus carrying out the mineralisation. During osteomalacia, calcification rate is decreased while the bone surface is being increased due to the building up of non-mineralised osteoid. The common symptoms of osteomalacia involves brittle bone, weakened muscles along with severe body ache [71–73]. Another known disease caused by pathological mineralisation is fibrous osteodystropy. In various findings, it has been seen that the flexibility and deformation of bone depends because of constant and extreme contact with the PTH, thus hampering with the bone load bearing capacity [74–78]. In osteocalcia, bone tissue is destroyed and resorped osteoclasts where the remodelling area, previously taken up by calcified bone is then occupied by fibrous connective tissue. As the disease progresses, non-mineralised formed bone takes up the place of cortical bone. And also, mineralised osteoid in the remodelling space was previously filled up by osseous tissue [79]. Paget's disease is a very commonly known disorder of bone in adults which is chronic in nature. This is also known as osteitis deformans [80, 81]. This is mainly found in middle aged men as compared to women [80, 82, 83]. In this disease, the resorption mechanism of bone gets speed up which results in formation of thick unarranged bone mineral matrix. As a result, producing weakened bone structure, painful fragile bone, joints arthritis inside the targeted bone. Sometimes, Paget's disease transform into a preliminary cancer of bone identified as Paget's sarcoma. The large multinucleated osteoclasts initiate the pathological Mineralisation of bones with high resorption causing Paget's disease [84]. This hastened resorption results in an unorganised deposition of mineral by osteoblasts during remodelling [85]. Thus causing irregularities in cortical thickness, coarsening of trabecular and vascularization of fibrous tissues, which then produces fatigue during high stress condition [81, 86, 87]. Another very well-known bone disease is osteoporosis, where the affected bone has depleted mass with structural degradation as well as amplified porosity of bone tissue [88]. Usually, in osteoporosis all bones are affected as compared to the Paget's disease where only a part of bone is targeted. When the mineral content of the bone goes below the critical value, the bone becomes more brittle in nature thus the load bearing capacity along with other mechanical properties of bone gets deteriorated [89]. The BMD also known as bone mineral density is directly proportional to the mechanical strength of bone. The patients with ongoing history of osteoporosis has reduced BMD, hence are more prone to fracture [90–93]. There are basically three main reasons behind the cause of osteoporosis. The first reason postulates that the commencing of the osteoporosis may be due to the underdevelopment of bone during the growth period of individuals. The second reason focuses on the bone development due to heightened resorption process.

And lastly, osteoporosis may happen due to the lack of new bone growth during the course of remodelling [94]. Earlier, it was believed that osteoporosis is an outbreak of ageing process but with the recent studies it has been observed that it may be caused because of malnutrition, alterations in biomechanical loading, production of excessive hormones and prolonged history of acidosis [95].

6. Mineralisation of synthetic biomaterials

For many years, biomaterials are being experimented in such a way so as to choose them as a replacement for damaged or diseased tissues. As the exclusive qualities of bone tissues are solely connected to the bone mineralisation, thereby interpreting and regulating the mineralisation process of artificial bone replacement is very crucial [20].

6.1 Inorganic materials

Since the mineral part of the bone is inorganic in nature, many biomaterials such as calcium phosphates, hydroxyapatite and bioglass are being used as bone alternatives [96]. It was in 1969, it was observed that bioglass forms bond with the bone thus restricting the development of fibrous tissues surrounding the bone [97]. There are various other ceramic biomaterials available such as hydroxyapatite [98], β-tricalcium phosphate, glass–ceramic [99–101], which are after sintering have displayed the bone bonding ability, hence paving the way for themselves clinically in reconstructive and regenerative medicine fields [102].

6.2 Calcium phosphates

The first in the list of very commonly used bioceramics are the calcium phosphate based ceramics, broadly used in the field of dentistry and orthopaedics. They are mainly used for purpose of coatings onto the top surface of metal implants such as titanium, etc. The study of mineralisation can be performed in some fluids which imitates the ionic composition of blood plasma. One such kind of fluid is simulated body fluid suggested by Kokubo et al. in 1991. This fluid mimics the ionic concentrations of human blood plasma almost exactly. This fluid is used for the purpose of studying mineralisation behaviour of biomaterials in vitro. The SBF initiates the formation of apatite (bone-like) over the synthetic biomaterial surfaces, thus giving the idea of mineralisation of the biomaterial in vivo [103]. Kokubo et al. also suggested that in vivo bone reaction of an artificial biomaterial can be anticipated by the formation of apatite over its surface in SBF, but this theory now has been challenged [104]. In 2010, Bertazzo et al. suggested that calcium phosphates exhibited the osteoconductive nature of bone guided by bone tissues beside the biomaterial surface at the site of implantation orthotopically. Also, there are other calcium phosphate ceramics present which shows osteoinductive traits i.e. ability to form bone at implantation site ectopically. Although, the exact mechanism behind the osteoinductive nature of certain calcium phosphates depend on various factors such as composition, structure and conformation of the calcium phosphate biomaterial [105].

6.3 Bioactive glasses

In 1969, Hench observed that certain silicon-based glasses formed bond with the bone [106] and named it as Bioactive® glass also known as 45S5 consisting of

45 wt% of SiO_2, 24.5 wt% of CaO, 24.5 wt% of Na_2O and 6 wt% of P_2O_5.it can be synthesised by two methods which are sol-gel and melt-quench. Because of its awesome response towards the bone formation in vivo, it has been considered for more clinical applications in the arena of orthopaedics. To comprehend the behaviour of bioglasses in vivo, they have been submerged in SBF to analyse the physio-chemical route of Mineralisation onto the material surface. It has been found that a carbonated apatite layer is formed which is almost like bone mineral. The mineralisation behaviour of calcium phosphate ceramics and bioglasses follow the same mechanism for the apatite formation, thus establishing a bond (chemically) between the host bone and biomaterial [107].

6.4 Organic materials

Moreover, along with inorganic biomaterials there are other organic biomaterial in the form of biopolymers are available which also shows excellent properties to be considered for tissue regeneration. Such kind of polymers is PLA (polylactic acid), PGA (polyglycolic acid), collagen, hyaluronic acid and many more. The biopolymers do not help in the formation of bone as that of bioceramics but they act as a supporting matrix for the treatment of damaged bone. The biopolymers are broadly divided into two categories I.e. Hydrated and non-hydrated polymers depending on the water retention ability [20].

6.5 Hydrated biopolymers

Hydrogels are considered under this label as the water is taken up by the polymeric network making them swollen in shape. Since they can take up water very quickly and can retain it, they are considered mainly for the application of cell culture, drug delivery and tissue engineering. On the other hand, they cannot be considered for bone regeneration purpose as they cannot provide any mechanical stability to the affected site [108, 109]. Also, the water content of hydrogels gets so high that it becomes almost impossible to sterilise them [110]. Furthermore, they fail to develop any with the surrounding tissues. The researchers around the world are trying to initiate mineralisation in the hydrogels by incorporating bioceramic particles such as calcium phosphates, hydroxyapatite or bioglass. Also, introduction of enzymes which catalyses the mineralisation activity into the hydrogels or injecting certain artificial analogues to the matrix vesicles in order to trigger biominerlisation. And, lastly the polymeric hydrogels are charged with negative ions or groups in order to invite positively charged calcium ions and thus stimulating mineralisation in inert hydrogels [110].

6.6 Non-hydrated biopolymers

This category includes many biopolymers such as PLA, PGA, collagen, chitosan, etc. to be considered broadly in the field of tissue engineering. The scaffolds which are made up of non-hydrated polymers can be developed by various techniques such as 3D printing [111], porogen leaching [112], fibre meshing, microsphere sintering [111], phase separation gas foaming [113], and supercritical fluid processing [114]. The scaffold so developed by aforementioned processing techniques differ in surface properties and porosities [115]. Mineralisation of these polymers can be attained by various ways such as incubating in SBF, modifying surface with anionic groups so as to attract calcium ions over the surface of biomaterial. In non-hydrated polymers the assessment of mineralisation behaviour can be studied using SBF similar to bioceramic scaffolds. The nature of SBF taken for mineralisation activity

impacts the ionic composition and the configuration of mineral phase for example more concentrated form of SBF speeds up the bone mineralisation. The negatively charged proteins play integral role in controlling the deposition of mineral phase onto the natural bone, thus affecting the mineralisation in SBF. Hence, the surface of biopolymer is functionalized with negatively charged proteins or bone related proteins so as to trigger biominerlisation [116–120].

7. Conclusions

Bone is a very complex organ which has hierarchical organised tissue that helps in giving protection and support mechanically to all the organs including brain because of its mineralised behaviour. The mineralisation of bone is mediated by either collagen or matrix vesicles. Both the pathways are interconnected; but, their interconnectivity is still not extensively studied. By studying the mineralisation pattern of bone, several bone related diseases caused due to pathological mineralisation or other reasons lacking in the mineralisation can be cured with the help of development of advanced biomaterials which may showcase equivalent levels of biological functioning when compared to the natural bone.

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References

[1] Burr DB. Bone morphology and organization. In: Basic and Applied Bone Biology. 2nd ed. Academic Press, Elsevier; 2019

[2] Bonnucci E, Motta PM. Ultrastructure of skeletal tissues. In: Bone and Cartilage in Health and Disease. Boston: Kluwer Academic Publishers; 1990

[3] Brookes M, Revell WJ. Blood Supply of Bone: Scientific Aspects. London: Springer-Verlag; 1998

[4] Burr DB, Allen MR. Calcified tissue international, special issue: Bone material properties and and skeletal fragility. Calcified Tissue International. 2015;**97**:199-241

[5] Castañeda-Corral G, Jimenez-Andrade JM, Block AP, Taylor RN, Mantyh WG, Kaczmarska MJ. The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A. Neuroscience. 2011;**178**:196-207

[6] Dempster D, Felsednberg D, van der Geest S. The Bone Quality Book. Amsterdam: Elsevier; 2006

[7] Enlow DH, Brown SO. A comparative histological study of fossil and recent bone tissues. Part III. Mammalian bone tissues. Texas Journal of Science. 1957;**10**:187-230

[8] Fonseca H, Moreira-Gonçalves D, Appell Coriolano HJ, Duarte JA. Bone quality: The determinants of bone strength and fragility. Sports Medicine. 2014;**44**:37-53

[9] Foote JS. A contribution to the comparative histology of the femur. Smithsonian Contrib. Knowl. 1916;**35**:1-242

[10] Fuchs RK, Allen MR, Ruppel ME, Diab T, Phipps RJ, Miller LM. In situ examination of the time-course for secondary mineralization of Haversian bone using synchrotron Fourier transform infrared microspectroscopy. Matrix Biology. 2008;**27**:34-41

[11] Fukumoto TJ. Bone as an endocrine organ. Trends Endocrinol. Metab. 2009;**20**:230-236

[12] Gurkan UA, Akkus O. The mechanical environment of bone marrow: A review. Annals of Biomedical Engineering. 2008;**36**:1978-1991

[13] Jee WSS, Weiss L. The skeletal tissues. In: Weiss L, editor. Histology: Cell and Tissue Biology. New York: Elsevier Biomedical; 1983

[14] Kaplan FS, Hayes WC, Keaveny TM, et al. Form and function of bone. In: Simon SR, editor. Orthopaedic Basic Science. Chicago: American Academy of Orthopaedic Surgeons; 1994

[15] Karsenty G, MacDougald O, Rosen CJ. Interactions between bone, adipose tissue and metabolism. Bone. 2012;**50**(Special Issue):429-579

[16] Martin RB, Burr DB, Sharkey NA, Fyhrie DP. Skeletal Tissue Mechanics. 2nd ed. New York: Springer-Verlag; 2015

[17] Reznikov N, Bilton M, Lari L, Stevens MM, Kröger R. Fractual-like hierarchical organization of bone begins at the nanoscale. Science. 2018;**360**:507-517

[18] Ruppel ME, Miller LM, Burr DB. The effect of the micro¬scopic and nanoscale structure on bone fragility. Osteoporos. Int. 2008;**19**:1251-1265

[19] Sivaraj KK, Adams RH. Blood vessel formation and function in bone. Development. 2016;**143**:2706-2715

[20] An J, Leeuwenburgh S, Wolke J, Jansen J. Mineralization processes in hard tissue: Bone. In: Biomineralization and Biomaterials. Elsevier; 2016. pp. 129-146

[21] Sommerfeldt D, Rubin C. Biology of bone and how it orchestrates the form and function of the skeleton. European Spine Journal. 2001;**10**(2):S86-S95

[22] Compton JT, Lee FY. A review of osteocyte function and the emerging importance of sclerostin. The Journal of Bone and Joint Surgery. American Volume. 2014;**96**(19):1659-1668

[23] Ferrari SL et al. A role for N-cadherin in the development of the differentiated osteoblastic phenotype. Journal of Bone and Mineral Research. 2000;**15**(2):198-208

[24] Harada S-I, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature. 2003;**423**(6937):349-355

[25] Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000;**289**(5484):1504-1508

[26] Walker DG. Enzymatic and electron microscopic analysis of isolated osteoclasts. Calcified Tissue Research. 1972;**9**(1):296-309

[27] Lanyon L. Osteocytes, strain detection, bone modeling and remodeling. Calcified Tissue International. 1993;**53**(1):S102-S107

[28] Bonewald LF. Osteocytes as dynamic multifunctional cells. Annals of the New York Academy of Sciences. 2007;**1116**(1):281-290

[29] Kamioka H, Honjo T, Takano-Yamamoto T. A three-dimensional distribution of osteocyte processes revealed by the combination of confocal laser scanning microscopy and differential interference contrast microscopy. Bone. 2001;**28**(2):145-149

[30] Baylink D et al. Vitamin D-enhanced osteocytic and osteoclastic bone resorption. The American Journal of Physiology. 1973;**224**(6):1345-1357

[31] Heino TJ, Hentunen TA, Väänänen HK. Conditioned medium from osteocytes stimulates the proliferation of bone marrow mesenchymal stem cells and their differentiation into osteoblasts. Experimental Cell Research. 2004;**294**(2):458-468

[32] Zhao S et al. MLO-Y4 osteocyte-like cells support osteoclast formation and activation. Journal of Bone and Mineral Research. 2002a;**17**(11):2068-2079

[33] Beniash E. Biominerals—Hierarchical nanocomposites: The example of bone. Wiley Interdisciplinary Reviews - Nanomedicine and Nanobiotechnology. 2011;**3**(1):47-69

[34] Boskey AL. Matrix proteins and mineralization: An overview. Connective Tissue Research. 1996;**35**(1-4):357-363

[35] Boskey AL. Biomineralization: Conflicts, challenges, and opportunities. Journal of Cellular Biochemistry. 1998;**72**(S30-31):83-91

[36] Hunter GK, Goldberg HA. Modulation of crystal formation by bone phosphoproteins: Role of glutamic acid-rich sequences in the nucleation of hydroxyapatite by bone sialoprotein. The Biochemical Journal. 1994;**302**:175-179

[37] Stubbs JT et al. Characterization of native and recombinant bone sialoprotein: Delineation of the mineral-binding and cell adhesion domains and structural analysis of the RGD domain. Journal of Bone and Mineral Research. 1997;**12**(8):1210-1222

[38] Gebauer D, Völkel A, Cölfen H. Stable prenucleation calcium carbonate clusters. Science. 2008;**322**(5909):1819-1822

[39] Pouget EM et al. The initial stages of template-controlled $CaCO_3$ formation revealed by cryo-TEM. Science. 2009;**323**(5920):1455-1458

[40] Chai YC et al. Current views on calcium phosphate osteogenicity and the translation into effective bone regeneration strategies. Acta Biomaterialia. 2012;**8**(11):3876-3887

[41] Anderson HC. Molecular biology of matrix vesicles. Clinical Orthopaedics and Related Research. 1995;**314**:266-280

[42] Anderson HC. Matrix vesicles and calcification. Current Rheumatology Reports. 2003;**5**(3):222-226

[43] Landis W. Chemistry and Biology of Mineralized Tissues. Toronto: University of Toronto Press; 2005

[44] Aparicio S et al. Optimal methods for processing mineralized tissues for Fourier transform infrared microspectroscopy. Calcified Tissue International. 2002;**70**(5):422-429

[45] Boskey AL. Mineralization of bones and teeth. Elements. 2007;**3**(6):385-391

[46] Palmer LC et al. Biomimetic systems for hydroxyapatite mineralization inspired by bone and enamel. Chemical Reviews. 2008;**108**(11):4754-4783

[47] He G et al. Phosphorylation of phosphophoryn is crucial for its function as a mediator of biomineralization. The Journal of Biological Chemistry. 2005;**280**(39):33109-33114

[48] Monfoulet L et al. Bone sialoprotein, but not osteopontin, deficiency impairs the mineralization of regenerating bone during cortical defect healing. Bone. 2010;**46**(2):447-452

[49] Farbod K et al. Interactions between inorganic and organic phases in bone tissue as a source of inspiration for design of novel nanocomposites. Tissue Engineering. Part B, Reviews. 2013;**20**(2):173-188

[50] Meyer U et al. Decreased expression of osteocalcin and osteonectin in relation to high strains and decreased mineralization in mandibular distraction osteogenesis. The Journal of Cranio-Maxillofacial Surgery. 1999;**27**(4):222-227

[51] Hoang QQ et al. Bone recognition mechanism of porcine osteocalcin from crystal structure. Nature. 2003;**425**(6961):977-980

[52] Roach H. Why does bone matrix contain non-collagenous proteins? The possible roles of osteocalcin, osteonectin, osteopontin and bone sialoprotein in bone mineralisation and resorption. Cell Biology International. 1994;**18**:617-628

[53] Rosenthal AK et al. Osteopontin promotes pathologic mineralization in articular cartilage. Matrix Biology. 2007;**26**(2):96-105

[54] Miller BF et al. Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. The Journal of Physiology. 2005;**567**(3):1021-1033

[55] Orgel JP et al. Microfibrillar structure of type I collagen in situ. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(24):9001-9005

[56] Nudelman F et al. The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. Nature Materials. 2010;**9**(12):1004-1009

[57] Glimcher M, Muir H. Recent studies of the mineral phase in bone and its possible linkage to the organic matrix by protein-bound phosphate bonds. Philosophical Transactions of the Royal Society B. 1984;**304**(1121):479-508

[58] Landis W et al. Mineral and organic matrix interaction in normally calcifying tendon visualized in three dimensions by high-voltage electron microscopic tomography and graphic image reconstruction. Journal of Structural Biology. 1993;**110**(1):39-54

[59] Traub W, Arad T, Weiner S. Origin of mineral crystal growth in collagen fibrils. Matrix. 1992;**12**(4):251-255

[60] Cölfen H. Biomineralization: A crystal-clear view. Nature Materials. 2010;**9**(12):960-961

[61] Stewart T. The presence of delayed hypersensitivity reactions in patients toward cellular extracts of their malignant tumors. 1. The role of tissue antigen, nonspecific reactions of nuclear material, and bacterial antigen as a cause for this phenomenon. Cancer. 1969;**23**(6):1368-1379

[62] Wu L et al. Induction of mineral deposition by primary cultures of chicken growth plate chondrocytes in ascorbate-containing media. Evidence of an association between matrix vesicles and collagen. The Journal of Biological Chemistry. 1989;**264**(35):21346-21355

[63] Hunter GK, Goldberg HA. Nucleation of hydroxyapatite by bone sialoprotein. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**(18):8562-8565

[64] Stechschulte Jr, DJ, et al. Presence and specific concentration of carbonic anhydrase II in matrix vesicles. Bone and Mineral. 1992;**17**(2):187-191

[65] Hirschman A et al. Neutral peptidase activities in matrix vesicles from bovine fetal alveolar bone and dog osteosarcoma. Calcified Tissue International. 1983;**35**(1):791-797

[66] Wuthier RE. The role of phospholipids in biological calcification: Distribution of phospholipase activity in calcifying epiphyseal cartilage. Clinical Orthopaedics and Related Research. 1973;**90**:191-200

[67] Dean DD et al. Matrix vesicles are enriched in metalloproteinases that degrade proteoglycans. Calcified Tissue International. 1992;**50**(4):342-349

[68] Wu L et al. Collagen-binding proteins in collagenase-released matrix vesicles from cartilage. Interaction between matrix vesicle proteins and different types of collagen. The Journal of Biological Chemistry. 1991;**266**(2):1195-1203

[69] Kirsch T. Determinants of pathological mineralization. Current Opinion in Rheumatology. 2006;**18**(2):174-180

[70] Yeni Y, Brown C, Norman T. Influence of bone composition and apparent density on fracture toughness of the human femur and tibia. Bone. 1998;**22**(1):79-84

[71] Parfitt A. Osteomalacia and related disorders. In: Metabolic Bone Disease and Clinically Related Disorders. 2nd ed. Philadelphia, PA: WB Saunders; 1990. pp. 329-396

[72] Feng JQ et al. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. Nature Genetics. 2006;**38**(11):1310-1315

[73] Aaron J et al. Frequency of osteomalacia and osteoporosis in fractures of the proximal femur. Lancet. 1974;**303**(7851):229-233

[74] Lynch MJ et al. Fibrous osteodystrophy in dromedary camels

(Camelus dromedarius). Journal of Zoo and Wildlife Medicine. 1999;**30**:577-583

[75] Fincham J et al. Mseleni joint disease. Part I. An animal model? South African Medical Journal. 1985;**67**(2):51-57

[76] Long GG et al. Fibrous osteodystrophy in an opossum. Journal of Wildlife Diseases. 1975;**11**(2):221-223

[77] Flom JO, Brown RJ, Jones RE. Fibrous osteodystrophy in a wild dolphin. Journal of the American Veterinary Medical Association. 1978;**173**(9):1124-1126

[78] Jaffe H, Bodansky A, Blair JE. Fibrous osteodystrophy (osteitis fibrosa) in experimental hyperparathyroidism of Guinea-pigs. Archives of Pathology. 1931;**11**:207

[79] Fetter A, Siemering G, Riser W. Osteoporosis and osteopetrosis. In: Newton CD, Nunamaker DM, editors. Textbook of Small Animal Orthopaedics. Philadelphia, PA: Lippincott; 1985. pp. 627-629

[80] Whyte MP. Paget's disease of bone. The New England Journal of Medicine. 2006;**355**(6):593-600

[81] Siris ES. Paget's disease of bone. Journal of Bone and Mineral Research. 1998;**13**(7):1061-1065

[82] Kanis JA. Pathophysiology and Treatment of Paget's Disease of Bone. Oxford: Taylor & Francis; 1998

[83] Altman RD et al. Influence of disodium etidronate on clinical and laboratory manifestations of Paget's disease of bone (osteitis deformans). The New England Journal of Medicine. 1973;**289**(26):1379-1384

[84] Rebel A et al. Osteoclast ultrastructure in Paget's disease. Calcified Tissue Research. 1976;**20**(1):187-199

[85] Meunier PJ et al. Bone histomorphometry in Paget's disease: Quantitative and dynamic analysis of pagetic and nonpagetic bone tissue. Arthritis and Rheumatism. 1980;**23**(10):1095-1103

[86] Merkow R, Lane J. Paget's disease of bone. The Orthopedic Clinics of North America. 1990;**21**(1):171-189

[87] Ooi C, Fraser W. Paget's disease of bone. Postgraduate Medical Journal. 1997;**73**(856):69-74

[88] Kanis J et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporosis International. 2007;**18**(8):1033-1046

[89] Gao H et al. Materials become insensitive to flaws at nanoscale: Lessons from nature. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(10):5597-5600

[90] Faibish D, Ott SM, Boskey AL. Mineral changes in osteoporosis: A review. Clinical Orthopaedics and Related Research. 2006;**443**:28

[91] Cefalu CA. Is bone mineral density predictive of fracture risk reduction? Current Medical Research and Opinion. 2004;**20**(3):341-349

[92] McCalden RW, McGeough JA. Age-related changes in the compressive strength of cancellous bone. The relative importance of changes in density and trabecular architecture. The Journal of Bone and Joint Surgery. American Volume. 1997;**79**(3):421-427

[93] Koh L, Ng D. Osteoporosis risk factor assessment and bone densitometry–current status and future trends. Annals of the Academy of Medicine, Singapore. 2002;**31**(1):37-42

[94] Chiappelli F. Osteoimmunopathology: Evidence-Based Perspectives from Molecular Biology to Systems Biology. Dordrecht: Springer; 2011

[95] NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA. 2001;**285**(6):785-795

[96] Jarcho M. Calcium phosphate ceramics as hard tissue prosthetics. Clinical Orthopaedics and Related Research. 1981;**157**:259-278

[97] Hench LL et al. Bonding mechanisms at the interface of ceramic prosthetic materials. Journal of Biomedical Materials Research. 1971;**5**(6):117-141

[98] Jarcho M et al. Tissue, cellular and subcellular events at a bone-ceramic hydroxylapatite interface. Journal of Bioengineering. 1977;**1**(2):79-92

[99] Ducheyne P, De Groot K. In vivo surface activity of a hydroxyapatite alveolar bonesubstitute. Journal of Biomedical Materials Research. 1981;**15**(3):441-445

[100] LeGeros R et al. Biphasic calcium phosphate bioceramics: Preparation, properties and applications. Journal of Materials Science. Materials in Medicine. 2003;**14**(3):201-209

[101] Kitsugi T et al. Bone bonding behavior of three kinds of apatite containing glass ceramics. Journal of Biomedical Materials Research. 1986;**20**(9):1295-1307

[102] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. 2006;**27**(15):2907-2915

[103] Kokubo T. Bioactive glass ceramics: Properties and applications. Biomaterials. 1991;**12**(2):155-163

[104] Bohner M, Lemaitre J. Can bioactivity be tested in vitro with SBF solution? Biomaterials. 2009;**30**(12):2175-2179

[105] Bertazzo S et al. Hydroxyapatite surface solubility and effect on cell adhesion. Colloids surf. B Biointerfaces. 2010;**78**(2):177-184

[106] Hench LL, Wilson J. Surface-active biomaterials. Science. 1984;**226**(4675):630-636

[107] Gross U, Schmitz HJ, Strunz V. Surface activities of bioactive glass, aluminum oxide, and titanium in a living environment. Annals of the New York Academy of Sciences. 1988;**523**(1):211-226

[108] Hoffman AS. Hydrogels for biomedical applications. Advanced Drug Delivery Reviews. 2002;**54**(1):3-12

[109] Driessens F et al. Effective formulations for the preparation of calcium phosphate bone cements. Journal of Materials Science. Materials in Medicine. 1994;**5**(3):164-170

[110] Gkioni K et al. Mineralization of hydrogels for bone regeneration. Tissue Engineering. Part B, Reviews. 2010;**16**(6):577-585

[111] Sherwood JK et al. A three-dimensional osteochondral composite scaffold for articular cartilage repair. Biomaterials. 2002;**23**(24):4739-4751

[112] Mikos AG et al. Preparation of poly(glycolic acid) bonded fiber structures for cell attachment and transplantation. Journal of Biomedical Materials Research. 1993;**27**(2):183-189

[113] Langer RS, Vacanti JP. Preparation of three-dimensional fibrous scaffold for attaching cells to produce vascularized tissue in vivo. Google Patents; 1998

[114] Ginty PJ et al. Mammalian cell survival and processing in supercritical CO_2. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(19):7426-7431

[115] Stevens MM. Biomaterials for bone tissue engineering. Materials Today. 2008;**11**(5):18-25

[116] Tanahashi M, Matsuda T. Surface functional group dependence on apatite formation on self-assembled monolayers in a simulated body fluid. Journal of Biomedical Materials Research. 1997;**34**(3):305-315

[117] Stephansson SN, Byers BA, García AJ. Enhanced expression of the osteoblastic phenotype on substrates that modulate fibronectin conformation and integrin receptor binding. Biomaterials. 2002;**23**(12):2527-2534

[118] Thorwarth M et al. Bioactivation of an anorganic bone matrix by P-15 peptide for the promotion of early bone formation. Biomaterials. 2005;**26**(28):5648-5657

[119] Filmon R et al. Effects of negatively charged groups (carboxymethyl) on the calcification of poly(2-hydroxyethyl methacrylate). Biomaterials. 2002;**23**(14):3053-3059

[120] Song J, Malathong V, Bertozzi CR. Mineralization of synthetic polymer scaffolds: A bottom-up approach for the development of artificial bone. Journal of the American Chemical Society. 2005;**127**(10):3366-3372