Chapter 14
Hybrid Composite for Orthopedic Applications

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14.1 Introduction

Various biomaterials have been explored for designing engineered bone tissue, ranging from natural to materials synthetic materials as well as the combination of the two employing numerous fabrication techniques. These materials are expected to resemble the chemical composition and architecture of the natural human bone as well as providing sufficient biomechanical properties to withstand load once implanted in the patient's body. In order to mimic the complex bone structure, a novel engineered bone material could be designed by combining two or more materials, forming a hybrid composite material. Ideally, this hybrid composite material should be biocompatible, bioresorbable/ biodegradable over time, osteoinductive and osteoconductive. Furthermore, the hybrid composite material should enable the fabrication of 3D porous structures with sufficient porosity and interconnected pores to allow cell ingrowth, transport of nutrients and metabolic waste throughout and most importantly promoting vascularization. In order to fabricate a suitable hybrid composite for bone regeneration, one should firstly understand the natural bone formation and remodelling, bone-health problems, as well as limitations of current treatments and current approaches to accelerate bone remodelling. This chapter will provide an insight into these aaspects to enable the right selection of

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materials for the fabrication of functional hybrid composite materials for bone regeneration.

14.2 Brief Insights into Bone Biology

Bone is a dynamic and highly vascularized tissue which undergoes remodelling throughout the lifetime of an individual (Salgado et al. 2004; Stevens 2008a). Basically, bone tissue is comprised of two main components; (i) the inorganic mineralized phase, which forms 65–70% of the bone tissue and consists of carbonated hydroxyapatite (CHA), and (ii) non-mineralized organic phase, which forms 30–35% of bone tissue and is predominantly collagen type I and other organic proteins. The inorganic mineralized phase increases the stiffness and the compressive strength of the bone while, the tensile strength and flexibility of bone are provided by the organic non-mineralized phase (Viguet-Carrin et al. 2006; Balint and Cartmell 2012). Each of the bone components plays an important role in allowing bone (1) to protect vital internal organs, (2) to support locomotion by providing support and site of muscle attachment, (3) to ensure that the skeleton has sufficient load-bearing capacity, (4) to promote the generation of red and white blood cells for oxygenation and immunological protection of other tissues and (5) to act as mineral reservoir for calcium, phosphate, and other important ions.

In adult skeleton, bone tissue can be divided into two architectural forms namely, the cortical also known as compact or dense bone (around 80% of total skeleton) and trabecular also called cancelleous or spongy bone (around 20% of total skeleton). Cortical bone is the denser bone, consisting of parallel cylindrical units with 5–10% porosity. Cortical bone is primarily found in the shaft of long bones such as femur, tibia, fibula; and forms the outer shell around the cancellous bone at the end of joints and the vertebrae. In contrast, the cancellous bone acquires sponge-like honeycomb morphology, comprising of branching bars, plates, and rods of various sizes called trabeculae. The mechanical properties of cancellous bone are greatly dependent on its porosity and internal porous structure. Its porosity ranges from 50 to 90%, making its ultimate compressive strength and modulus of elasticity 10 times inferior compared to cortical bone. Besides that, the pores also perform other physiological functions and contain the marrow. Cancellous bone is normally found at the end of the long bones in vertebrate and in flat bones like the pelvis (Salgado et al. 2004; Stevens 2008a).

The formation, maintenance and resorption of bone tissue results from the interaction of bone cells, namely osteoblasts, osteocytes and osteoclasts. Osteoblasts are responsible for the production and mineralization of the bony matrix whereas, osteoclasts are responsible for bone resorption during remodelling, the repair of micro-damage and the adaptation to mechanical loading. Osteocytes are the most abundant cells in bone which are characterized by stellate shape and possess fewer organelles than the osteoblasts. Osteocytes reside in the lacunae within the mineralized matrix and act as the mechanosensor cells of bone (Balint and Cartmell 2012;

Kini and Nandeesh 2012). Bone development and repair are finely coordinated through the balance between bone matrix resorption and formation orchestrated by osteoclasts and osteoblasts, respectively. Together, they form multicellular units, present in the vicinity of the vascular spaces and bone surfaces, responsible for bone remodelling. Fundamentally, the bone remodelling process involves six stages, namely (1) quiescence, (2) activation, (3) resorption, (4) reversal, (5) formation and (6) mineralization (Kohli et al. 2018). The understanding of the physiological process involved in bone remodelling is of critical importance with regards to the design and development of a biomaterial for it to completely reconstruct the damaged tissue. The next section of this review will discuss the need of biomaterial intervention in accelerating bone remodelling followed by the sequence of events that take place upon biomaterial implantation.

14.3 Clinical Needs for Bone Regeneration

Bone loss or dysfunction due to disease, trauma or infection can dramatically alter one's body equilibrium and quality of life (Salgado et al. 2004; Balint and Cartmell 2012). It is considered as one of the major public health problems, which could result in huge socioeconomic implications. For younger people who generally have higher regenerative capacity, most fractures will possibly heal without the need of any intervention (Stevens 2008a). However, it is not the same scenario for elderly people. The growing elderly population is one of the major factors contributing to the increase in bone-related degenerative diseases such as osteoporosis, which is due to hormonal changes and oxidative stress related to aging. Proximal femur, proximal humerus or the vertebral body are the typical locations of such commuted fractures for these patients. The reconstruction of fractured bones remains a critical challenge in the field of orthopaedic surgery. According to the National Health Service's (NHS) records, more than 300,000 people receive hospital treatment for fragility fractures every year as a result of osteoporosis in the United Kingdom, thus resulting in enormous economic burden for the NHS. For instance, hip fractures alone cost the UK an estimated £5 million per day- that is approximately £2 billion pounds per year.

In other parts of the world, particularly in Asia, the largest bone-related are due to the high rate of road accidents (Muhammad et al. 2012). For instance, in Malaysia alone, annually there are over 400,000 road accidents with approximately 7000 fatal cases and over 10,000 serious and minor injuries. In 2014, it was reported that Malaysian roads were considered the 17th most dangerous roads in the world with road accidents leading to serious trauma and fractures. In addition to trauma the loss or resection of bone due to tumour or infection can cause critical-size defects (CSD). Bone-health problems do not only restrict the physical movement of the patients but indirectly also affect the psychological condition and quality of life of an individual (Balint and Cartmell 2012).

Historically, bone defects are treated autografts or allografts. Transplanting autologous bone has been considered as gold standard in clinic as it integrates

reliably with the host bone and avoids immune- and disease-related complications (Athanasiou et al. 1996). Allografts, on the other hand, are bone donated from other patients. However, numerous drawbacks of this treatment have been reported such as short supply, high cost, and donor site morbidity as associated with the harvest and potential risk of disease transmission. The limitations associated with the use of autografts and allografts have driven the development of various engineered bone biomaterials. The use of these biomaterials could reduce the risk of disease transmission, the number of surgical procedures, cost, pain and immunogenicity as well as eliminate the issue regarding the shortage of supply (Kohli et al. 2018; Fernandez de Grado et al. 2018). Various materials such as natural or synthetic biomaterials mostly based on calcium phosphates bioceramics, polymer-based substitutes and biological products such as growth factors have been developed for bone tissue engineering (BTE) applications. To date, no adequate bone substitute has been developed to meet the clinical needs; hence, bone-health problems remain unresolved. The search of new bone regeneration strategies is therefore a key priority fuelled by the debilitating pain associated with bone damage, particularly in managing large bone defects. For these particular defects, not only is the bone tissue damaged, but the surrounding vascular network is often markedly disrupted as well, which can consequently affect the repair response of the tissue. Bone regeneration is a complex process as it involves not only bone cells, vascular network but also the surrounding immune responses. Developing porous synthetic materials such as 3D hybrid composite biomaterials, which can ultimately act as bone scaffolds exhibiting bone like composition and architecture is crucial. These bone scaffolds are able to support faster bone regeneration and inclusion of functional vascular networks within their structure.

14.4 Approaches to Accelerate Bone Regeneration

With a long history of development, Calcium orthophosphates (CaPs) have been accepted as main inorganic components of hard tissues of vertebrates. The mineral phase of bone and teeth is a basic calcium phosphate, which is assimilated to synthetic hydroxyapatite (HA) with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. Besides its strong affinity for the mineral constituents of bones, HA also has good bioactivity, osteoconductivity, and biocompatibility with the human bone tissue. These properties provide a rationale for its use as bone substitute material in orthopaedic and dental applications. However, biological apatites differ from stoichiometric HA in several respects, including non-stoichiometry, small crystal dimensions and poor crystallinity (i.e. low degree of structural order) (Boanini et al. 2010; Malhotra and Habibovic 2016; Baba Ismail et al. 2017). Biological apatites are uniquely similar in that they all comprise carbonate (CO_3) in varying amounts of 2–8 wt %, preferentially substituting the PO_4 site (B-type) compared with OH (A-type) ions in the apatite lattice. The composition of CO_3 depends on bone age, site, sex, and health of the individual (Zhou et al. 2008; Landi et al. 2003).

For the past few decades, much research has demonstrated that a variety of trace elements such as carbonate, magnesium, zinc, strontium, and cobalt can be incorporated into the HA lattice to improve its properties by producing a mineral composition more akin to that of mineral native bone tissue. Not only the chemical composition of the biomaterials plays a major role, but also the ability to encourage rapid osteogenesis coupled with angiogenesis. These processes are intricately linked and osteogenesis would not be possible without angiogenesis (Grellier et al. 2009; Bose et al. 2013). A common way to promote osteoinductivity and agiogenesis in CaPs scaffolds is by incorporating growth factors such as recombinant human bone morphogentic protein-2 (rhBMP-2), transforming growth factor (TGF-β), insulin growth factor (IGF), vascular endothelial growth factor (VEGF) and a variety of bisphosphates (BPs)(Hankenson et al. 2011; Portal-Núñez et al. 2012). However, recent concerns over their safety has lead to increased resistance of their use through the Food and Drug Administration (FDA). The major concerns have related to ectopic or unwanted bone formation, which in certain situations can lead to very serious side effects (Boraiah et al. 2009; Luca et al. 2010). An alternative and potentially safer strategy has been the addition of multi-doping ions into the HA lattice as it has been reported that the addition of trace elements can lead to controlled resorbability, improve mechanical strength and positively influence the biological response (Bose et al. 2013; Bandyopadhyay et al. 2006). The roles of these trace elements and their mechanisms of action are summarized in Table 14.1.

14.5 Materials for Bone Scaffolds

Given the demanding clinical need, it is not surprising that the market for biomaterial-based orthopedic treatments is evolving at a rapid rate. While materials intended for the implantation in the classical approach were in the past designed to be bioinert, material scientists have now shifted toward the use of bioactive materials. These bioactive materials are supposed to integrate with the host and regenerate damaged. For BTE applications, these bioactive materials should preferably be osteoinductive, osteoconductive and osseointegrative. The terms osteoinductive, osteoconductive and osseointegrative are repeatedly used in many orthopaedic papers, but not always correctly defined. Thus, the suggested definitions of these terms are shown in Table 14.2.

The selection of the most appropriate material for the fabrication of a scaffold is very important, as its properties will influence the scaffold properties to a great extent. A number of materials such as metals, ceramics and polymers have been proposed but most metals and ceramics are non-biodegradable, which leaves the researcher's choice to limited small number of ceramics and biodegradable polymers.

Table 14.1 Roles of trace elements and their mechanisms of action

Trace elements	Role	Mechanism of action
CO_3^{2-}	Major substituent in bone	The presence of B-carbonate in the apatite lattice causes a decrease in crystallinity and increase in solubility in both *in vitro* and *in vivo* tests (Landi et al. 2003; Murugan and Ramakrishna 2006). The higher the carbonate content, the higher the metabolic activity of the tissue (Landi et al. 2010)
Sr^{2+}	Osteogenesis	Strontium stimulates osteoblasts activity and simultaneously inhibits sthe activity and differentiation of osteoclast cells (Aina et al. 2012; Chandran et al. 2016)
Co^{2+}	Angiogenesis	Cobalt ions can induce hypoxia on the cellular level. Cells compensate for this hypoxic environment by expressing genes (such as VEGF) that promote neovascularization and angiogenesis (Pacary et al. 2006; Kim et al. 2006a). In large doses, CO^{2+} can also induce toxicity. Increased soluble CO^{2+} ion levels might cause serious adverse reactions to the surrounding tissues as well as systematic toxicity (Simonsen et al. 2012).
Zn^{2+}	Osteogenesis	Zinc ions have a positive effect on bone metabolism. In the cellular microenvironment, Zn^{2+} are thought to promote osteoblastic bone formation and inhibit osteoclastic bone resorption *in vitro* (Bose et al. 2013; Dasgupta et al. 2010)
Mg^{2+}	Angiogenesis	Magnesium induces nitric oxide production in endothelial cells which is essentially the same function as VEGF uses to induce angiogenesis (Cooke 2002; Maier et al. 2004)

Table 14.2 Definitions of osteoinductive, osteoconductive and osseointegrative

Terms	Definitions
Osteoinductive	Capable to stimulate the differentiation of progenitor cells towards osteoblastic lineage
Osteoconductive	Permits bone growth on its surface and supports the ingrowth of surrounding bone
Osseointegrative	Ability to integrate into surrounding bone which forms a direct contact between host bones and implant

14.5.1 Bioactive Ceramics

Bioactive ceramic materials have similar composition to the inorganic mineral phase of bone, and hence hydroxyapatite (HA) and tricalcium phosphate (TCP) are of clinical interest (Best et al. 2008; Stevens 2008b). The rationale of using these calcium phosphate (CaP) based materials stems from the fact that CaP is the major component of biological apatite and that it shows promises of biocompatibility, osteoconductivity and biodegradability.

Synthetic HA has been used as coatings on metallic implants, fillers in polymer matrices and scaffolds for maxillofacial reconstruction, treatment of bone defects, total joint replacement and revision surgery for the last 20–30 years (Best et al. 2008). However, previous studies have reported that pure HA shows negligible

resorption even years after implantation. Besides, it was found that biological apatites differ chemically from stoichiometric HA in that they contain a number of additional trace elements substituted into the HA lattice (Boanini et al. 2010; Gibson and Bonfield 2002; David et al. 2013).

Back in 1960s, Raquel LeGeros (1969) first started the work on the characterization of carbonated HA (CHA) for biomedical application. Since then, synthetic CHA has been extensively studied, as carbonate is the most abundant substitution in bone mineral (2–8 wt%). Its amount depends on bone age, site, animal species and individual. Thus, biological apatite is more accurately described as carbonated HA rather than HA alone (Landi et al. 2003; Best et al. 2008; Merry et al. 1998; Tadic et al. 2002). There are three types of carbonate substitution; (i) the substitution of carbonate for hydroxyl ions (A-type), (ii) carbonate substitution for phosphate site (B-type) and (iii) both hydroxyl and phosphate groups substituted by carbonate (AB-type) (Landi et al. 2010; LeGeros et al. 1969; Shepherd et al 2012). Previous studies have shown that the presence of B-carbonate in the apatite lattice causes a decrease in crystallinity and increase in solubility in both *in vitro* and *in vivo* tests. The increase in solubility has considerably enhanced the bioactivity of CHA. This has been shown by greater bone apposition found around dense CHA compared to pure HA. Besides CHA, various other trace elements have been incorporated into the hydroxyapatite structure with the aim to either improve the osteogenesis (bone formation) or angiogenesis (vascular network formation). Among the trace elements that have been investigated and incorporated into the apatite structure are Sr^{2+}, Co^{2+}, Zn^{2+} and Mg^{2+}. Their roles are described in Table 14.1.

For all the aforementioned reasons, the development of synthetic HA powders with fully complete and controlled levels of ionic substitutions into the HA lattice seem promising candidates for the fabrication of ideal bone scaffold materials. These materials have the potential to become the new "gold standard", mimicking the composition of the natural human bone mineralized matrix (Sprio et al. 2008).

14.5.2 Biodegradable Polymers

There are two types of biodegradable polymers, i.e. natural and synthetic polymers (Chen et al. 2002; Rezwan et al. 2006). Natural polymers, such as collagen and hyaluronic acid are among the potential candidates for bone substitute materials which would provide essential biological guidance to cells, supporting cell attachment and promoting chemotactic responses. Collagen is the major component of extracellular matrix (ECM) which is responsible for cellular adhesion and proliferation (Kim et al. 2010; Zhao et al. 2014). It occurs in many places throughout our body. For instance, it is found in bone (Type I), cartilage (Type II), blood vessel walls (Type III), cell basement membrane (Type IV) and cell surfaces (Type V). Collagen type I is a popular choice of material for the scaffold preparation for bone regeneration since it offers excellent biocompatibility, degrades easily and is resorbed by the body. It also promotes cell attachment, but its mechanical properties

however, are much lower than that of the native bone (Wahl and Czernuszka 2006; Jones et al. 2010).

On the other hand, hyaluronic acid is the major non-collagenous component present in the ECM and the synovial fluid (Zhao et al. 2014; Zhang et al. 2005). Therefore, hyaluronic acid and collagen, have been incorporated into 3D scaffolds for bone regeneration (Holtorf et al. 2005; Yu et al. 2012). Hyaluronic acid and collagen type I have been used as coating materials for PLLA films to enhance cell-material interaction. These coatings may improve the bioactivity of PLLA films, for potential BTE applications (Zhao et al. 2014).

The most often utilized biodegradable synthetic polymers for 3D scaffolds in BTE are saturated poly-α-hydroxyl esters such as poly (lactic acid) (PLA), poly (glycolic acid) (PLGA), as well as poly (lactic-co-glycolide) (PLGA) copolyers (Athanasiou et al. 1996; El-amin et al. 2003; Li et al. 2010). These polymers have been approved by the FDA for certain human clinical use, such as surgical sutures and some implantable devices (Chen et al. 2001). The chemical properties of these polymers allow hydrolytic degradation through de-esterfication. The degradation by-products can be removed by the body through natural metabolic pathways as lactic and glycolic acids. The degradtion rate, physical and mechanical propeties of these polymers can be easily tailored over a wide range by using various molecular weights and copolymers. However, these polymers undergo bulk erosion process, where a massive release of the acidic by-products could cause local inflammatory reactions in vivo. Consequently, this can cause the scaffold to fail prematurely. Another drawback of these synthetic polymers is related to their hydrophobicity and lack of physiological activity (Kim et al. 2006b). It has been shown that PLA does not provide a favorable surface for cell attachment and proliferation due to lack of specific cell recognition sites. Modification of the outermost part of the material is seen to be sufficient as to tailor their biocompatibility, while the bulk properties of the materials are maintained.

14.5.3 Composites

Composite or hybrid materials can be generated via the combination of functional polymers with inorganic nanostructured compounds. Inorganic-organic composites aiming to closely mirror the composite nature of real bone by combining the toughness of a polymer phase with the compressive strength of a ceramic phase has been shown to improve both the degradation and mechanical properties of these hybrid scaffolds (Stevens 2008b). For instance, tissue-engineered HA-Collagen nanocomposite systems are developing rapidly and showing promise (Wahl and Czernuszka 2006; Jones et al. 2010). Comparing ceramic scaffolds and ceramic composite scaffolds, it was shown that HA-Collagen composite have better osteoinductive capacity compared to single HA or TCP. Several approaches have been developed to create the inorganic-organic polymer composites including blending, sol-gel and emulsion polymerization. The main challenge in synthesizing composite materials

to recreate the organization of native organic and inorganic components at the nanoscale as found in vivo. However, mechanical properties of these composites is still low compared to the native bone (Stevens 2008b).

14.6 Engineered Scaffolds for Bone Tissue Engineering

In seeking to meet the current challenges of hard tissue augmentation through mimicking the native bone, clinically viable three-dimensional multi-functional scaffolds can be incorporated as matrices for the regeneration of new tissue. Scaffolds can be used either as permanent or temporary template to restore organ functionality. To facilitate tissue repair, scaffolds should meet certain criteria, which might vary slightly between types of tissues. Nevertheless, the following properties have been identified as essential scaffold criteria for BTE applications:

(a) Scaffolds should be biocompatible, which suggests that they should be well integrated in the host's tissue without inducing any adverse response (Salgado et al. 2004; Rezwan et al. 2006). Scaffold should also be osteoconductive in order to guide the formation of new bone tissue along their surfaces (Leong et al. 2003).
(b) Scaffolds should be biodegradable/ bioresorbable. The scaffold should allowed tissues/cells to adhere, proliferate, and differentiate to form healthy tissues and help tissue recovers to the original shape and strength. Subsequently, the scaffold would then degrade while the tissue regenerates (Hutmacher 2000).
(c) Scaffolds should be osteoinductive to promote bone tissue regeneration in large bone defects. Natural osteoinduction in combination with a biodegradable scaffold may not be enough to facilitate bone healing (Albrektsson and Johansson 2001).
(d) Surface properties both chemical and topographical of a scaffold are primarily important to regulate cell activities, such as for adhesion, differentiation, proliferation and thus promote tissue growth (Oh et al. 2006).
(e) Scaffolds should possess sufficient amount of porosity (40–90% depends on the nature of biomaterials used) to allow cell ingrowth as well as flow of nutrients and metabolic waste throughout the entire scaffold (Hutmacher 2000).
(f) The pore size of a scaffold should be large enough to allow cell penetration. It is well recommended that for BTE application, the pore size should be in the range of 200–900 μm (Mikos and Temenoff 2000).
(g) Scaffolds should demonstrate adequate mechanical strength so that they do not collapse during handling and during the patient's daily activities. *In vitro*, the scaffolds should have sufficient strength to withstand the mechanical stimuli applied when cultured in dynamic 3D culture environments such as bioreators (Leong et al. 2003).

(h) Biomaterials should also be reproducible and processable into 3D scaffolds with various shapes and sizes (Leong et al. 2003; Hutmacher 2000). The fabrication process should be controllable and cost-effective (Hutmacher et al. 2004).
(i) As the scaffolds will be in direct contact with the biological environment, they should be easily sterilizable to prevent infection (Rezwan et al. 2006).
(j) Suitable substrate stiffness is also important because it has been shown to affect cell responses including proliferation, differentiation, migration and apoptosis while cells are being attached to a scaffold. It has a huge influence on cell migration, proliferation and apoptosis (Pelham and Wang 1998; Wang et al. 2000).

14.7 Conclusion and Future Direction

Key requirements for engineering a functional hybrid composite for bone regeneration are depending on the selection materials to be used, the appropriate ratio of inorganic and organic materials as well as the architectural design of the hybrid composite scaffolds. These influence the properties of the hybrid composite to a great extent. It is critically important to ensure the produced hybrid composite closely resembles the composition and architecture of natural human bone, and reaches a balance between mechanical and biological performance to assure successful bone regeneration.

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References

Aina V et al (2012) Magnesium- and strontium-co-substituted hydroxyapatite: the effects of doped-ions on the structure and chemico-physical properties. J Mater Sci Mater Med 23:2867–2879

Albrektsson T, Johansson C (2001) Osteoinduction, osteoconduction and osseointegration. Eur Spine J 10:S96–S101

Athanasiou KA, Niederauer GG, Agrawal CM (1996) Biocompatibility and clinical applications of polylactic acid / polyglycolic acid copolymers. 17

Baba Ismail YM, Wimpenny I, Bretcanu O, Dalgarno K, El Haj AJ (2017) Development of multisubstituted hydroxyapatite nanopowders as biomedical materials for bone tissue engineering applications. J Biomed Mater Res – Part A 105:1775–1785

Balint R, Cartmell SH (2012) Osteoblasts and their applications in bone tissue engineering. Cell Health and Cytoskeleton 4:49–61

Bandyopadhyay A, Bernard S, Xue W, Böse S (2006) Calcium phosphate-based resorbable ceramics: influence of MgO, ZnO, and SiO 2 dopants. J Am Ceram Soc 89:2675–2688

Best SM, Porter AE, Thian ES, Huang J (2008) Bioceramics: Past, present and for the future. *J. Eur. Ceram. Soc* 28:1319–1327

Boanini E, Gazzano M, Bigi A (2010) Ionic substitutions in calcium phosphates synthesized at low temperature. Acta Biomater 6:1882–1894

Boraiah S, Paul O, Hawkes D, Wickham M, Lorich DG (2009) Complications of recombinant human BMP-2 for treating complex tibial plateau fractures: a preliminary report. Clin Orthop Relat Res 467:3257–3262

Bose S, Fielding G, Tarafder S, Bandyopadhyay A (2013) Understanding of dopant-induced osteogenesis and angiogenesis in calcium phosphate ceramics. Trends Biotechnol 31:594–605

Chandran S, Suresh Babu S, Hari Krishnan VS, Varma HK, John A (2016) Osteogenic efficacy of strontium hydroxyapatite micro-granules in osteoporotic rat model. J Biomater Appl 31:499–509

Chen G, Ushida T, Tateishi T (2001) Development of biodegradable porous scaffolds for tissue engineering. Mater Sci Eng C 17(1–2):63–69

Chen, G., Ushida, T., Tetsuya, T. Scaffold Design for Tissue Engineering. (2002)

Cooke JP (2002) Nitric Oxide and Angiogenesis. *Circulation* 105:2133–2135

Dasgupta S, Banerjee SS, Bandyopadhyay A, Bose S (2010) Zn- and Mg-doped hydroxyapatite nanoparticles for controlled release of protein. Langmuir 26:4958–4964

David M et al (2013) Physico-chemical characterization and in vitro biological evaluation of pure SiHA for bone tissue engineering application. 530:351–356

El-amin SF et al (2003) Extracellular matrix production by human osteoblasts cultured on biodegradable polymers applicable for tissue engineering. 24:1213–1221

Fernandez de Grado G, Keller L, Idoux-Gillet Y, Wagner Q, Musset AM, Benkirane-Jessel N, Bornert F, Offner D (2018) Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. *J. Tissue Eng.* 9. https://doi.org/10.1177/2041731418776819

Gibson IR, Bonfield W (2002) Novel synthesis and characterization of an AB-type carbonate-substituted hydroxyapatite. J Biomed Mater Res 59:697–708

Grellier M, Bordenave L, Amédée J (2009) Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering. Trends Biotechnol 27:562–571

Hankenson KD, Dishowitz M, Gray C, Schenker M (2011) Angiogenesis in bone regeneration. Injury 42:556–561

Holtorf HL, Sikavitsas VI, Jansen JA (2005) Effect of bone extracellular matrix synthesized in vitro on the osteoblastic differentiation of marrow stromal cells. 26:971–977

Hutmacher DW (2000) Scaffolds in tissue engineering bone and cartilage. Biomaterials 21:2529–2543

Hutmacher DW, Sittinger M, Risbud MV (2004) Scaffold-based tissue engineering: rationale for computer added design and solid free-form fabrication systems. 2

Jones GL et al (2010) Primary human osteoblast culture on 3D porous collagen-hydroxyapatite scaffolds:1244–1250. https://doi.org/10.1002/jbm.a.32805

Kim KS, Rajagopal V, Gonsalves C, Johnson C, Kalra VK (2006a) A novel role of hypoxia-inducible factor in cobalt chloride- and hypoxia-mediated expression of IL-8 chemokine in human endothelial cells. J Immunol 177:7211–7224

Kim S-S, Park MS, Jeon O, Choi CY, Kim BS (2006b) Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. Scanning 27:1399–1409

Kim TG, Park S-H, Chung HJ, Yang D-Y, Park TG (2010) Microstructured scaffold coated with hydroxyapatite/collagen nanocomposite multilayer for enhanced osteogenic induction of human mesenchymal stem cells. J Mater Chem 20:8927

Kini U, Nandeesh BN (2012) Radionuclide and hybrid bone imaging. Radionucl Hybrid Bone Imaging 9783642024**,** 1–1046

Kohli N et al (2018) Bone remodelling in vitro : Where are we headed ? -A review on the current understanding of physiological bone remodelling and in fl ammation and the strategies for testing biomaterials in vitro. 110**,** 38–46

Landi E, Celotti G, Logroscino G, Tampieri A (2003) Carbonated hydroxyapatite as bone substitute. J Eur Ceram Soc 23:2931–2937

Landi E, Uggeri J, Sprio S, Tampieri A, Guizzardi S (2010) Human osteoblast behavior on as-synthesized SiO(4) and B-CO(3) co-substituted apatite. J Biomed Mater Res A 94:59–70
LeGeros RZ, Trautz OR, Klein E, LeGeros JP (1969) Two types of carbonate substitution in the apatite structure. Experientia 25:5–7
Leong KF, Cheah CM, Chua CK (2003) Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. Biomaterials 24:2363–2378
Li J et al (2010) A one-step method to fabricate PLLA scaffolds with deposition of bioactive hydroxyapatite and collagen using ice-based microporogens. Acta Biomater 6:2013–2019
Luca L, Rougemont AL, Walpoth BH, Gurny R, Jordan O (2010) The effects of carrier nature and pH on rhBMP-2-induced ectopic bone formation. J Control Release 147:38–44
Maier JAM, Bernardini D, Rayssiguier Y, Mazur A (2004) High concentrations of magnesium modulate vascular endothelial cell behaviour in vitro. Biochim Biophys Acta Mol basis Dis 1689:6–12
Malhotra A, Habibovic P (2016) Calcium phosphates and angiogenesis: implications and advances for bone regeneration. Trends Biotechnol 34:983–992
Merry JC, Gibson IR, Best SM, Bonfield W (1998) Synthesis and characterization of carbonate hydroxyapatite. J Mater Sci Mater Med 9:779–783
Mikos AG, Temenoff JS (2000) Formation of highly porous biodegradable scaffolds for tissue engineering 3:1995–2000
Muhammad H, Khairuddin NA, Zakaria AR, NNN Y, Wan Mustafa WM (2012) Facial fractures presenting to a tertiary referral centre in Malaysia: A 9-year study. *Malays. Dent. J.* 34:10–15
Murugan R, Ramakrishna S (2006) Production of ultra-fine bioresorbable carbonated hydroxyapatite. Acta Biomater. 2:201–206
Oh S, Oh N, Appleford M, Ong JL (2006) Bioceramics for tissue engineering applications – a review. Am J Biochem Biotechnol 2:49–56
Pacary E et al (2006) Synergistic effects of CoCl(2) and ROCK inhibition on mesenchymal stem cell differentiation into neuron-like cells. J Cell Sci 119:2667–2678
Pelham RJ, Wang YL (1998) Cell locomotion and focal adhesion are regulated by substrate flexibility. Proceeding National Academy of Sciences USA, 94:13661–13665
Portal-Núñez S, Lozano D, Esbrit P (2012) Role of angiogenesis on bone formation. Histol Histopathol 27:559–566
Rezwan K, Chen QZ, Blaker JJ, Roberto A (2006) Biodegradable and bioactive porous polymer / inorganic composite scaffolds for bone tissue engineering. 27:3413–3431
Salgado J, Coutinho OP, Reis RL (2004) Bone tissue engineering: state of the art and future trends. Macromol Biosci 4(8):743–765
Shepherd JH, Shepherd DV, Best SM (2012) Substituted hydroxyapatites for bone repair:2335–2347. https://doi.org/10.1007/s10856-012-4598-2
Simonsen LO, Harbak H, Bennekou P (2012) Cobalt metabolism and toxicology-a brief update. Sci Total Environ 432:210–215
Sprio S et al (2008) Physico-chemical properties and solubility behaviour of multi-substituted hydroxyapatite powders containing silicon. 28:179–187
Stevens MM (2008a) Biomaterials for bone materials tissue engineering. Mater Today 11:18–25
Stevens MM (2008b) Biomaterials for bone Materials that enhance bone regeneration have a wealth of potential. 11:18–25
Tadic D, Peters F, Epple M (2002) Continuous synthesis of amorphous carbonated apatites. 23:2553–2559
Viguet-Carrin S, Garnero P, Delmas PD (2006) The role of collagen in bone strength. Osteoporos Int 17:319–336
Wahl DA, Czernuszka JT (2006) Collagen-Hydroxyapatite composites for hard tissue repair. 11:43–56
Wang HB et al (2000) Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. Am J Physiol Cell Physiol 279:C1345–C1350

Yu B et al (2012) Effect of calcium source on structure and properties of sol − gel derived bioactive glasses.
Zhang J et al (2005) Natural polyelectrolyte films based on layer-by layer deposition of collagen and hyaluronic acid. Biomaterials 26:3353–3361
Zhao MY, Li LH, Li B, Zhou CR (2014) LBL coating of type I collagen and hyaluronic acid on aminolyzed PLLA to enhance the cell-material interaction. 8:322–335
Zhou WY, Wang M, Cheung WL, Guo BC, Jia DM (2008) Synthesis of carbonated hydroxyapatite nanospheres through nanoemulsion. J Mater Sci Mater Med 19:103–110