Calcium phosphate bioceramics for improved angiogenesis

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9.1 Introduction

During the last decades, there has been an excessive need for artificial bone tissue substitutes across the globe. The main reasons for such demand are associated with the increased aging population, obesity, cancers, accidents, and congenital genetic abnormalities. Accordingly, several attempts have been made to design and develop potent replacements capable of accelerating bone tissue healing. Previously, the specific and strict criteria have been defined by governmental agencies and regulatory bodies (e.g., Food and Drug Administration [FDA] and International Organization for Standardization [ISO], respectively) for any substance that should be implanted into the body. From a clinical point of view, the biocompatibility of materials is of utmost importance and defined through a series of well-established assays (e.g., cytotoxicity, hemocompatibility, etc.). However, biomaterials designed to repair and reconstruct damaged bones should meet other criteria, both structurally and functionally. In general, osteoconduction, osteoinduction, and osteointegration are stated as ideal biological properties of bone substitutes. Osteoconductive materials provide a proper substrate for bone growth on their surface. Osteoinductive materials can induce osteogenesis (new bone formation) through the recruitment of osteogenic cells to the injured region. Osteointegration means the ability of an exogenous material to incorporate within the bone and strongly attach to the surrounding bone [1].

Calcium phosphates (CaPs) represent versatile materials for biomedical applications, including bone tissue reconstruction. Hydroxyapatite (HAp) and beta-tricalcium phosphate (β- TCP) are two well-known members of the CaPs bioceramics family. It has been shown that CaPs and their constructs possess mechanical properties (e.g., compressive strengths) closely in the range of human bones. Still, the low strength, high brittleness, as well as limited bioactivities prohibit the extensive usage of CaPs bioceramics in many load-bearing applications [2,3]. Another disadvantage of CaPs is attributed to their poor degradation rate that inhibits bone ingrowth in implants and suppresses osteogenesis [3]. CaPs, predominantly in the form of ion-substituted

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HAp, form the inorganic phase of bone tissues; therefore, they have been extensively investigated for bone repair and regeneration. Though, the solubility of CaPs is probably one of the most important characteristics that should be controlled as it may interface with the tissue healing process. Prior experiments have shown that CaPs can properly support the adhesion and proliferation of bone-forming cells (e.g., osteoblasts) in vitro and in vivo [4]. Thus, CaPs bioceramics (e.g., HAp) are generally recognized as osteoconductive biomaterials [5]. Although the osteoinductive properties of CaPs are attributed to their microstructural surface properties (grain size, microporosity, surface roughness, and specific surface area), this feature is not well-identified via in vivo animal studies [6,7]. In addition to improving osteogenesis, specific types of CaPs bioceramics showed the capability of inducing neovessel formation (angiogenesis). It has currently become a scientific fact that materials having proangiogenic activity can be more effective for accelerating wound healing as regards newly formed blood vessels are responsible for providing nutrients and oxygens to cells and removing waste metabolites from the injured site [8,9]. Respecting this critical issue, we aim to provide substantial information on the proangiogenic potential of CaPs bioceramics and its importance in developing of novel therapies for bone tissue engineering.

9.2 CaPs bioceramics: An overview

CaPs bioceramics have a long history in medicine with huge numbers of successfully performed clinical studies. Indeed, CaPs represent the main inorganic constituents of bone tissues in the body [10]. Hence, they are extensively applied for managing hard tissue defects. However, recent publications have declared specific types of CaPs could be used for treating soft tissue injuries [11]. These substances exhibit remarkable compatibility with the living systems (cells, tissues, and organs) and their by-products cause no adverse effects in the human body [10,12]. CaPs are generally bioresorbable compounds that can be replaced with natural tissue during the bone healing process. The dissolution rate of CaPs can greatly affect their biological functions (e.g., osteogenesis); therefore, a series of physico-chemical methods have been utilized to tune CaPs boiceramics properties. One of the most promising approaches is to dope metallic and nonmetallic elements to the CaPs network for generating potent substances in terms of osteogenesis and angiogenesis. For instance, lithium (Li), zinc (Zn), strontium (Sr), and magnesium (Mn) doped CaPs have exhibited improved osteogenesis while doping of copper (Cu) and cobalt (Co) to CaPs make them proangiogenic materials [13, 14]. It worth mentioning that some elements (e.g., boron (B) and silicon (Si)) can simultaneously improve osteogenic and angiogenic properties of CaPs [15]. Surface functionalization of CaPs is recognized as another approach applied for improving their biological activities. In this regard, surface properties of CaPs, including surface charge and roughness, can be optimized by physico-chemical modification methods. Experimental data have clarified that surface-modified CaPs can facilitate cellular interactions and improve cell adhesion, proliferation, and differentiation [16]. In addition to affecting biological properties, the defects could change the

physico-chemical properties of CaPs, including band-gap, surface charge, surface area, and ions release/uptake [17,18].

The inherent properties, including crystallographic and thermodynamic parameters, of commonly used CaPs in tissue engineering approaches are shown in Table 9.1. As it can be seen in Table 9.1, the ratio of Ca/P strongly influences the crystallographic parameters. Structural features and atomic distribution of CaPs were proven to be influential in determining their biological performance.

9.3 Clinical significance of angiogenesis in bone regeneration

In the body, blood vessels are responsible for providing oxygen and nutrients to cells and tissues as well as removing waste materials. The vasculature is obviously found in connective tissues like the bone [19]. The vasculature network of the bone is predominantly formed via angiogenesis. Developmental biology studies specify that the bone formation and vascularization processes concomitantly take place and can proceed through either endochondral ossification or intramembranous ossification. The vast majority of bones, including long bones (e.g., the femur), are generated by the endochondral ossification. On the other side, flat bones (e.g., the skull bones) are formed by using the intramembranous ossification in which mesenchymal cells are directly differentiated into osteoblasts. It should be mentioned that flat bones consist of a layer of compact bone interspersed with bone marrow [20]. In addition to skeletal development, the formation of new blood vessels is crucial during fracture healing [21]. Immediately after the damage, the volume of the vascular bed of bone tissue significantly increases due to the vasodilation process. This is followed by the formation of fracture hematoma, serving as a template for the development of the provisional, vascular bone callus [22]. The newly formed blood vessels may create other benefits for the bone healing process as to their angiocrine function (secreting paracrine signals) that is useful for coordinating of growth and differentiation of osteogenic progenitor cells [23].

Experimental data indicates that diverse molecular mediators (e.g., growth factors and cytokines) govern highly orchestrated interactions between different cells (macrophages, mesenchymal stem cells, etc.) to form new mineralized bone tissue [24,25]. Among different molecular mediators, vascular endothelial growth factor-A (VEGF-A) is known as a master regulator of bone development and regeneration (Fig. 9.1) [26]. In fact, these molecules can promote the migration and proliferation of endothelial cells (ECs) as well as stimulate the osteogenesis process through the regulation of osteogenic GFs [27,28]. During bone tissue repair, VEGF is generally produced by osteoblasts and enhances the ECs' migration and proliferation [29]. It should be mentioned that the physiological level of VEGF is strictly regulated as its low values may interrupt osteoblast differentiation, and while its high values increase osteoclast recruitment, leading to bone resorption [22].

Table 9.1 A summary of crystallographic and thermodynamic parameters of calcium phosphates (CaPs) [12].

Compound	Formula	Ca/P ratio	Space group	Unit cell parameters (Å and °)	Z	Density ($g.cm^3$)	pH stability in aqueous solution (298.15 K)	Thermodynamic parameters (298.15 K)		
								H° kJ/mol	S° J/(mol·K)	C_p
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2{\cdot}H_2O$	0.5	Triclinic Pī	a = 5.6261(5), b = 11.889(2), c = 6.4731(8) $\alpha = 98.633(6)$ $\beta = 118.262(6)$ $\gamma = 83.344(6)$	2	2.23	0.0 ± 2.0	−3410.0	259.8	165.0 + 318T
Monocalcium phosphate anhydrate (MCPA)	$Ca(H_2PO_4)_2$	0.5	Triclinic Pī	a = 7.5577(5), b = 8.2531(6), c = 5.5504(3) $\alpha = 109.87(1)$, $\beta = 93.68(1)$, $\gamma = 109.15(1)$	2	2.58	–	−3115.0	189.5	259.8
Dicalcium phosphate dihydrate (DCPD, "brushite")	$CaHPO_4 \cdot 2H_2O$	1	Monoclinic Ia	a = 5.812(2), b = 15.180(3), c = 6.239(2) $\beta = 116.42(3)$	4	2.32	2.0 ± 6.0	−2421.2	189.5	225.9 + 55T
Dicalcium phosphate anhydrate (DCPA, "monetite")	$CaHPO_4$	1	Triclinic Pī	a = 6.910(1), b = 6.627(2), c = 6.998(2) $\alpha = 96.34(2)$, $\beta = 103.82(2)$, $\gamma = 88.33(2)$	4	2.89	–	−1814.4	111.4	138.4 + 55T
Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$	1.33	Triclinic Pī	a = 19.692(4), b = 9.523(2), c = 6.835(2) $\alpha = 90.15(2)$, $\beta = 92.54(2)$, $\gamma = 108.65(1)$	1	2.61	5.5−7.0	−13375.2	878.22	883.1
α-Tricalcium phosphate (α-TCP)	α-$Ca_3(PO_4)_2$	1.5	Monoclinic $P2_1/a$	a = 12.887(2), b = 27.280(4), c = 15.219(2) $\beta = 126.20(1)$	24	2.86	–	−4109.9	240.9	231.6
β-Tricalcium phosphate (β-TCP)	β-$Ca_3(PO_4)_2$	1.5	Rhombohedral R3Ch	a = b = 10.439(1), c = 37.375(6) $\gamma = 120$	21	3.07	–	−4120.8	236.0	227.8
Amorphous calcium phosphate (ACP)	$Ca_x(PO_4)_y \cdot nH_2O$	1.2−2.2	–	–	–	–	–	–	–	–
Calcium-deficient hydroxyapatite (CDHA)	$Ca_{10\text{-}x}(HPO_4)_x(PO_4)_{6\text{-}x2\text{-}x}$ (0<x<1)	1.5−1.67	–	–	–	–	6.5--9.5	–	–	–
Hydroxyapatite (HA)	$Ca_{10}(PO_4)_{62}$	1.67	Monoclinic $P2_1/b$	a = 9.84214(8), b = 2a, c = 6.8814(7) $\gamma = 120$	4	3.16	9.5--12	–	–	–
			Hexagonal $P6_3/m$	a = b = 9.4302(5), c = 6.8911(2) $\gamma = 120$	2			−13477.0	780.7	963.5 + 108.2T
Tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$	2	Monoclinic $P2_1$	a = 7.023(1), b = 11.986(4), c = 9.473(2) $\beta = 90.90(1)$	4	3.05	–	−4940.6	275.5	271.5

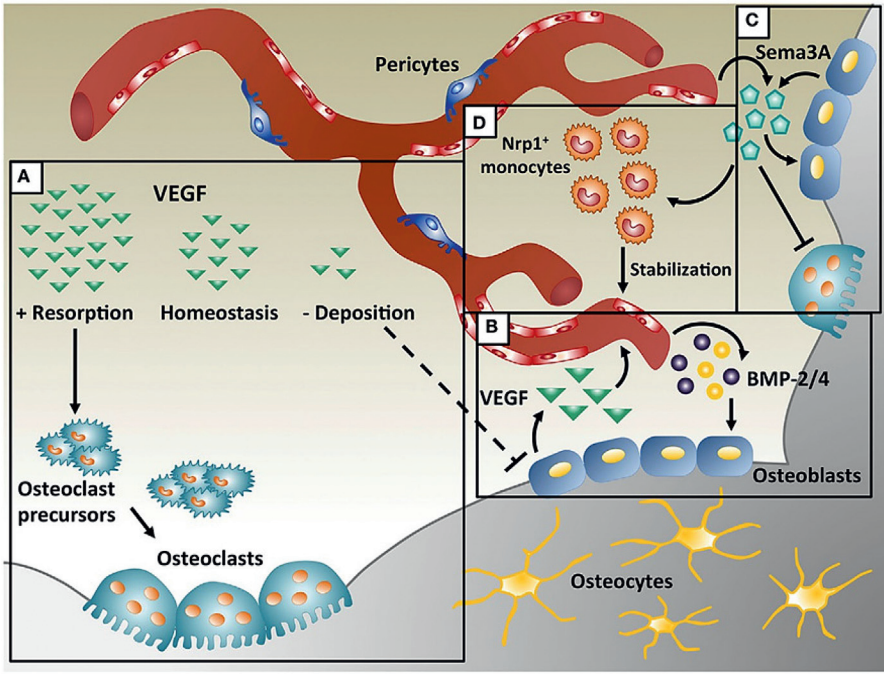

Figure 9.1 Schematic representation displaying the coupling of angiogenesis and osteogenesis during intramembranous ossification. (A) Vascular endothelial growth factor (VEGF) at physiological levels guarantees bone homeostasis. (B) During bone repair, VEGF secreted by osteoblasts promotes the migration and proliferation of endothelial cells (ECs). In turn, ECs secrete osteogenic factors (e.g., bone morphogenetic protein (BMP)-2 and -4) and thereby support osteoblast differentiation. (C) VEGF can regulate the expression of semaphorin 3A (Sema3A) in ECs in a dose-dependent manner; Sema3A suppresses osteoclast differentiation and induces bone deposition. (D) Sema3A is also identified as the main mediator for the recruitment of neuropilin 1-expressing (Nrp1 +) monocytes, which improve vessel stabilization. Reproduced from [22].

9.4 CaPs bioceramics for improved angiogenesis

Tissue healing is a complicated process in which well-orchestrated biological events happen for obtaining a timely repair. In the case of bone tissue, osteoconductivity, osteoinductivity and osseointegration are regarded as three main features defined for any ideal substitute to achieve a perfect healing process [30]. Previously reported research indicates that CaPs (HAp, TCP, biphasic CaPs (BCP = HAp + TCP), etc.) are among the osteoconductive biomaterials [4]. In addition, CaPs are known suitable substances for bone tissue engineering due to their capability of enhancing osteogenesis. It is well-recognized that failing to provide sufficient nutrients and oxygen to cells at damaged sites may result in failure in tissue transplantation. Therefore, numerous attempts have been made to produce CaPs with higher angiogenic capacity in the last decade.

Currently, the proangiogenic capability of CaPs-based tissue substitutes takes into account the meantime of designing and preparing the final products. Different types and forms of CaPs have shown the ability to improve neovascularization [31]. Several parameters can determine the proangiogenic capacity of CaPs, including their chemical formulation and physical properties. In this regard, the role of physical characteristics was stated as a key determinant in the angiogenic potential of CaPs ceramics. For illustration, TCP with submicron surface topography could more effectively stimulate M1 to M2 macrophage polarization and thereby enhance the secretion of proangiogenic growth factor and chemokine TGF-β and CCL18 as compared with the samples having micron-scale topography [32]. The in vivo results confirmed that the intramuscular implantation of TCPs with submicron surface topography into adult male beagles could increase typical M2 macrophage markers (e.g., IL-10), leading to enhance blood vessel formation and improved bone formation. Prior studies have clarified that positive effects of CaPs on new blood vessel formation can even be observed after making composite constructs with them. In this sense, octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$) included in gelatin could trigger the capillary-like tube formation in human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner [33]. Some innovative approaches were developed over the last decades to improve the proangiogenic potential of CaPs ceramic, including doping of proangiogenic elements in their chemical composition as well as surface modification approaches. These strategies are introduced and discussed in the following sections based on the recent publications.

9.4.1 Doped CaPs bioceramics for promoted angiogenesis

There are several trace elements (e.g., copper) in the human body that are needed for mediating a wide range of biochemical reactions. Previously, biological impacts of these metallic elements have been entirely studied through in vitro or in vivo assays [34,35]. Improving cell proliferation, providing antibacterial and anti-inflammatory properties, as well as promoting angiogenesis are some of the most well-known biological features rendered by administrating trace elements [36]. Therefore, adding trace elements to the structure of CaPs ceramics has become a routine approach in the biomedical setting to positively affect the tissue repair and regeneration processes [37]. In fact, doped-CaPs can be served as drug delivery vehicles for providing a sustained release of therapeutic ions at damaged sites. Concerning neovascularization, specific kinds of trace metallic elements (e.g., copper and cobalt) may be incorporated into CaPs structure to promote angiogenesis either in vitro or in vivo (Table 9.2).

The introduction of proangiogenic metallic dopants to CaPs ceramics was confirmed as a safe and efficient strategy in favor of tissue engineering. It should be emphasized that this process may alter the physico-chemical, mechanical and biological properties of CaPs [38]. Silicon (Si), copper (Cu), cobalt (Co), and boron (B) are among the most well-studied elements for inducing angiogenesis [39]; they could be easily incorporated into CaPs structure and make a proangiogenic substitute for accelerating wound healing. Silicon is generally recognized as a semi-metal material (metalloid) with some critical functions in the human body. Silicate ions (SiO_4^{4-}) can play a direct

Table 9.2 A summary of calcium phosphates (CaPs) ceramics doped with proangiogenic elements for potential use in bone tissue engineering.

CaPs ceramics	Dopants	Remarks	Ref
60 wt% TCP, 25 wt% dicalcium phosphate anhydrous (DCPA), 10 wt% HAp nanoparticles, and 5 wt% calcium carbonate (CC)	Copper at dosages of 0.01, 0.05, 0.1 and 0.5 wt%	The 0.01 wt% Cu-doped enhanced: - The proliferation of bone marrow mouse bone marrow stromal cells (mBMSCs) - The expression of osteogenic differentiation-related genes (collagen I and osteocalcin) and proteins (ALP and collagen I). - The proliferation of HUVECs - The expression of angiogenesis-related genes (eNOs, VEGF and bFGF), proteins (VEGF and NO) - *in vitro* tube formation of HUVECs	[36]
HAp/ β-TCP with a ratio of 1.67	Silicon at dosages of 0.1, 0.4 mol %	The 0.1 mol% Si^{4+} doped samples could: - Increase *in vitro* bone nodule formation - improve cell migration rate	[45]
Brushite cement (Ca/P ratio of $\sim$ 1.5) composited with silk	Silicon and zinc at dosages of 0.5 and 0.25 wt %, respectively	The Si/Zn doped samples could: - Increase the nitric oxide (NO) secretion by endothelial cells - Overexpress hypoxia-inducible factor 1 alpha (HIF-1α) - Enhance tube formation	[48]
CaP-coated poly (D,L-lactic acid) (PLA)	Cobalt at dosages of 0.1 and 20 μM	– 0.1 μM Co^{2+} doped CaPs could: - Improve the upregulation of HIF-1α *in vitro* - Generate greater blood vessel area and higher numbers of large blood vessels in vivo	[58]
β-TCP/HAp scaffolds containing growth differentiation factor-5 (GDF-5)-loaded poly(lactide-co-glycolide) (PLGA) microspheres	Copper and zinc at dosages of 0.005, 0.02, and 0.05 M	The Cu/Zn-doped composites could: - Improve the proliferation of BMSCs and vascular endothelial cells - Enhance the VEGF secretion	[76]

role in the mineralization of bones during the later stages of calcification [40]. The proangiogenic activity of silicate ions has been previously demonstrated; they were extensively applied in tissue engineering strategies for obtaining accelerated wound healing. The presence of silicates can induce neovessel formation by upregulating endothelial nitric oxide synthase (eNOS), leading to increased production of a series of proangiogenic growth factors (VEGF, bFGF, and TGF-b) [41]. In addition, these ions may increase the Ca^{2+} levels in cells and thereby affect the proliferation and motility of vascular ECs in favor of sprout outgrowth of new blood vessels [42–44]. Accordingly, Si-doped CaPs were developed, and reported data indicated superior biological properties of these materials for tissue repair and regeneration applications as compared to their un-doped counterparts [45]. As previously stated by Prof. Bohner, apart from passive mechanisms (change in the grain size and protein conformation at the material surface) that describe biological effects of Si substitution in materials, an active mechanism, i.e., Si release, is responsible for positive changes in cellular behaviors [46,47]. The proangiogenic activities of Si-doped CaPs were even observed after adding to a polymeric substrate both in vitro and in vivo, holding promises in bone tissue engineering [48].

Copper (Cu) is known as an essential cofactor in humans and shows desirable therapeutic properties for managing different types of disorders and diseases. On this matter, the antibacterial activity of Cu was recently reviewed, and its usefulness in orthopedic applications has been highlighted [49]. On the other side, Cu was approved for its biological effectiveness in activating several growth factors, cytokines, and molecules involved in advancing the angiogenesis process, including VEGF, FGF-1 and -2, fibronectin, angiogenin [50]. Cu^{+}/Cu^{2+} ions were demonstrated to be effective in the initiation, maturation, and regulation of blood vessel formation (ECM remodeling). Cu may promote angiogenesis via two specific signaling pathways of (I) the hypoxia-inducible HIF-1 and (II) the mitogen-activated protein kinase (MAPK) pathways [51]. Cu-containing CaPs bioceramics (e.g., Cu-doped HAp and β-TCP) have been successfully synthesized and demonstrated for their ability to promoting neovessel formation [35,52,53]. For instance, an improved angiogenesis was observed after the incubation of HUVECs with 0.01 wt% and 0.05 wt% Cu-doped calcium phosphate cement [36]. Additionally, the fabrication of 3D scaffolds from Cu-doped ceramics has also been evaluated for potential use in bone tissue engineering strategies [54,55]. As cutting-edge research, designing and developing of 3D printed scaffolds made of Cu-doped CaPs may open new horizons in the orthopedic setting with huge opportunities.

Cobalt is another proangiogenic trace element in the human body, which is normally found at the center of vitamin B12 and some other co-enzymes (named cobalamins). Although cobalt is generally recognized as a toxic element, it can be used for medical purposes at very low concentrations without any adverse effects. Divalent cobalt ions (Co^{2+}) could stimulate neovascularization through specific signaling pathways; for example, they mimic hypoxia conditions via the stabilization of hypoxia-inducible factor-1 alpha (HIF-1α), which lead to the upregulation of proangiogenic factors VEGF and bFGF [56,57]. Adding cobalt into different types of CaPs bioceramics was previously investigated, and reported data have been quite promising in terms of improved angiogenesis capacity [58]. As an illustration, β-TCP powders doped

with different concentrations of cobalt (0, 2, 5 mol%) showed the ability to increase VEGF expression in human bone marrow mesenchymal stem cells (HBMSCs) as well as the capacity of the tube-like structure formation in HUVECs. The findings clarified that the substitution of cobalt with calcium in the β-TCP structure might also lead to suppressing the phase transition from β- to α-TCP [59]. Cobalt-substituted HAp powders are another successful example of proangiogenic CaPs ceramics; 0.33% (w/w) Co^{2+} doping to HAp network resulted in stabilizing HIF-1α and consequently enhanced production of VEGF in human osteoblast-like cells (MG-63) [60]. There are several studies indicating the effectiveness of three-dimensional (3D) constructs made of Co^{2+}-substituted CaPs ceramics in bone tissue engineering [61]. Still, there is a scientific gap in designing and developing 3D printed constructs of cobalt-substituted CaPs bioceramics for potential use in hard tissue engineering. Still, the main concern on using Co^{2+}-doped CaPs bioceramics is associated with the risk of toxic effects of cobalt for living systems. Accordingly, cautions should be taken in the case of in vivo implantation of cobalt-containing CaPs. In addition, the sustained release of Co^{2+} ions from CaPs-based scaffolds should be carefully considered as a critical factor in determining biological outcomes.

Boron (B) is a nonmetal micronutrient in the human body that plays varying vitally important roles in a wide range of biological functions (e.g., bone metabolism). The level of this trace element in human bone tissue is about 0.90 ppm and is regarded as a major factor in preventing skeletal loss and protecting the organism from the development of osteoporosis [62,63]. Considering its biological properties, boric acid (H_3BO_3) at low concentrations can activate the MAPK signaling pathway, leading to improved cell growth and proliferation [64]. Furthermore, H_3BO_3 can stimulate the translation of mRNAs encoding proangiogenic proteins, including VEGF and transforming growth factor β (TGF-β) [65]. Therefore, this trace element has been considered in proangiogenic strategies for years; B-containing biomaterials were developed as new types of tissue substitutes capable of accelerating wound healing [66,67]. The substitution of borate groups into CaPs ceramics may happen on PO_4 and OH sites, predominately the first [68]. B-doped biphasic HAp/β-TCP samples were previously synthesized and characterized for potential use in bone tissue engineering [69]. Moreover, the in vitro osteogenic capacity of composite scaffolds based on B-doped HAp and chitosan was previously reported [70]. However, there is a noticeable shortcoming in experimental studies on utilizing and investigating B-substituted CaPs bioceramics for improved angiogenesis. It is worth noting that the incorporation of nanoplatforms of B (e.g., nanotubes) into CaPs bioceramics was also evaluated, and reported data has been promising for stimulated new bone formation [71]. Still, the proangiogenic activity of such compositions has not well-studied either by in vitro or in vivo assays.

In addition to the elements mentioned above, a couple of chemicals with proangiogenic capacity were also substituted into the CaPs network for promoting their inherent biological properties in terms of neovascularization [72,73]. Zinc (Zn) is among the most promising elements exhibiting proangiogenic activities through upregulation of relevant growth factors like VEGF and FGF [74,75]. This element, either alone or in combination with other elements, can be easily incorporated into CaPs structure and applied for tissue engineering applications [34]. It should be stated that the proangiogenic

potential of most of these elements in CaPs is dose-dependent. All in all, although the primary results of CaPs bioceramics substituted with proangiogenic dopants have been quite promising in terms of tissue engineering applications, some critical points remain to be solved. For example, achieving a sustained release of dopants during a long period seems necessary with regard to the tissue repair timeline. This will be even more complicated if doped CaPs apply for managing injuries and damages related to other tissues (e.g., dental). The second limitation may correlate to the necessary amounts of each proangiogenic dopant in CaPs structure. High concentrations of some trace elements could cause cellular and molecular damages and thereby hinder the healing process. For example, although cobalt may promote new blood vessel formation, cautions should be taken into accounts as it is a toxic element at specific dosages for the living system.

9.4.2 Functionalized CaPs for induced angiogenesis

Experimental studies have demonstrated that surface properties, including chemical composition and surface structure, could play an important role in the biological properties of biomaterials [77]. In addition, the surface can be considered an appropriate place to graft bioactive molecules and improve their biological performance, such as angiogenesis capability [78]. Up to now, several surface modification techniques were well developed and applied to generate more suitable substitutes for tissue regenerations applications, including grafting, coating, and so on [79]. Physical adsorption of a proangiogenic GF (i.e., VEGF) to CaPs bone cement Biocement D was previously reported effective for activating angiogenesis by sustained delivery of VEGF [80]. The importance of small molecules and oligonucleotides is clearly understood in inducing or suppressing biological reactions in mammalian cells [81]. There are some interesting reports in the literature in which CaPs bioceramics have been armored with these therapeutic molecules and applied for obtaining accelerated tissue healing. For instance, the 3R02 bivalent DNA aptamer was immobilized onto HAp particles by using 3-aminopropyltriethoxysilane (APTES), and obtained in vivo data indicated that the particles are suitable candidates for enhancing angiogenesis and osteogenesis as regards their ability to capture higher VEGF protein and enhance the growth of HUVECs compared to pristine counterparts [82]. It should be highlighted that the use of linkers (e.g., peptide linkers) maybe as a beneficial for the grafting and immobilizing of different molecules onto CaPs surface at the same time in defined ratios [83]. Prior experiments have well-proved that specific sorts of plant extracts can activate molecular signaling pathways involved in angiogenesis in a dose-dependent manner. This issue can be acknowledged as a unique opportunity for designing novel proangiogenic CaPs in a green route.

9.5 CaPs bioceramics for delivery of proangiogenic bioactive molecules

CaPs bioceramic are generally identified as suitable vehicles for loading and delivery of a wide range of chemicals and bioactive molecules (proteins, nucleic acids, and so

on) [84]. In fact, CaPs nanoparticles can provide an increase in the local drug levels in the desired areas and decrease the risks of systemic toxicity [85]. Additionally, achieving a prolonged drug release is feasible by CaPs ceramics, ranging from insoluble, hydrophobic or hydrophilic drugs. This feasibly is also practicable in the case of CaPs-based composites [86]. The possibility to make stimuli-responsive delivery systems by CaPs particles is considered outstanding merit for their applications in biomedicine. For example, thermo- and pH-sensitive CaPs ceramics were previously developed and successfully applied for biomedical approaches [87,88].

Focusing on the angiogenesis issue, CaPs ceramics in different shapes and forms have been investigated for loading and delivery of proangiogenic cargoes [89]. For instance, mesoporous HAp microspheres were successfully utilized as a sustained release system for delivery of simvastatin to improve osteogenesis and angiogenesis and consequently accelerated bone regeneration (Fig. 9.2) [90]. Furthermore, researchers were succeeded in developing injectable CaPs foams based on TCP with the capability of loading and delivery of Pitavastatin as an osteogenic and angiogenic agent [91]. This system could serve as a delivery platform for the controlled release of Pitavastatin and enhanced in vitro mineralization and vascularization in a dose-dependent manner. It is worth mentioning that in vivo degradation of CaPs ceramics can be regarded as a beneficial parameter in providing a sustained release profile of proangiogenic molecules. On this matter, TGF-β1 adsorbed to β-TCP was previously reported to have the ability to induce neovascularization and enhance bone formation [92].

It should be mentioned that the pore size and porosity of CaPs-based scaffolds were previously introduced as important determinants affecting bone formation and angiogenesis [93]. Scaffold porosity was reported as a more dominant player in promoting bone formation and neovascularization in comparison with the pore size. Though, the release rate and release fraction of osteogenic and proangiogenic growth factors were mostly controlled by the scaffold's pore size. In addition to conventionally fabricated constructs, 3D printed CaPs bioceramic implants are also utilized for loading and delivery of proangiogenic growth factors (e.g., VEGF) with promising reported data on their effectiveness in promoted neovascularization and wound healing [94,95]. Still, the current knowledge on this issue should be extended by performing more experimental studies, especially those are associated with in vivo studies.

9.6 Summary and future perspectives

Calcium phosphates (CaPs) are among the most promising materials for managing broad ranges of skeleton systems. Importantly, the main inorganic phase of native bone is made of calcium and phosphates elements, predominantly in the form of ion-substituted Ca-deficient hydroxyapatite crystals. That is why CaPs bioceramics exhibit inherent properties in favor of bone tissue regeneration, including excellent biocompatibility, bioactivity and osteoconductivity. In recent decades, understanding the real capacity of CaPs ceramics in stimulating angiogenesis, that is, new blood vessel formation has been one of the hottest topics in materials science and bioengineering fields. It is well-known that the degree of bone repair directly depends on

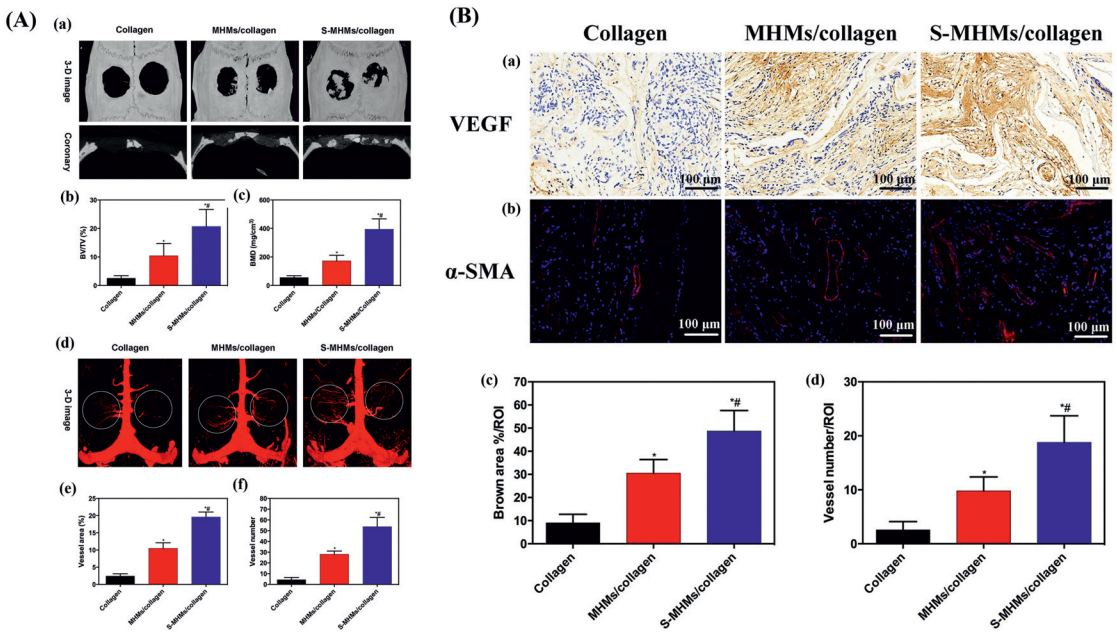

Figure 9.2 (A) Three dimensional (3-D) and coronary views of reconstructed calvaria bone treated by collage, mesoporous HAp microspheres (MHMs)/collagen, simvastatin-loaded MHMs (a); bone mineral density (BMD), and bone volume (BV)/total volume (TV) in the defect sits (b and c); newly formed blood vessels provided by 3-D reconstruction images; quantitative analysis of the new blood vessel area and number (e and f). (B) Immunohistochemistry evaluation of angiogenic markers in each group, including VEGF and α-SMA. *Comparison between collagen and other groups, #comparison between the MHMs/collagen and S-MHMs/collagen groups, $p < .05$). Reproduced with permission from [90].

the number of new blood vessels in the damaged region. In fact, blood vessels are responsible for providing sufficient levels of oxygen and nutrients to cells and tissues and remove their waste metabolites from the affected site. Accordingly, the design and preparation of proangiogenic CaPs can be useful for obtaining accelerated bone tissue healing. Scientific evidence indicates both physical characteristics and the chemical composition may affect the angiogenic potential of CaPs. On this matter, surface topography is recognized as a primary parameter in the proangiogenic activity of CaPs bioceramics. Understanding the ion release mechanism from CaPs structure led to the emergence of the term "drug delivery systems" for these materials. Currently, a series of proangiogenic elements, mostly metallic ions such as Cu^{2+} and Co^{2+}, doped to the CaPs network for making substances with more angiogenic capacity. In addition, CaPs provide an outstanding opportunity for loading and delivery of proangiogenic drugs and chemicals into injured regions for boosting bone tissue healing. In this regard, plant-derived components and phytochemicals with proangiogenic activity (e.g., curcumin) may be suitable candidates for developing angiogenesis-inducing CaPs bioceramics. The fabrication of 3D printed CaPs scaffolds with the ability to stimulate angiogenesis is regarded a step-forward for bone tissue engineering applications. Still, more research, especially clinical trials, is required to perfectly design and conduct for revealing all the pros and cons of angiogenesis-inducing CaPs in managing bone tissue lesions.

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Non-Print Items

Abstract

Calcium phosphate (CaP) bioceramics represent versatile biomaterials in the biomedical setting, especially for managing damaged hard tissues. Hydroxyapatite (HAp) and tricalcium phosphate (TCP) are among the most well-known CaPs bioceramics with diverse applications in tissue engineering. These substances offer exceptional characteristics for bone repair and regeneration, including excellent biocompatibility, osteoconductivity, osteoinductivity, and osteogenesis. The ability of CaPs to induce new blood vessel formation, that is, angiogenesis, is considered an additional advantage for their in vivo applications. The chemical composition and physical properties (e.g., topography) of CaPs bioceramics were identified as key determinants in advancing angiogenesis. Current research emphasizes making modifications in the basic formulation of CaPs for enhancing their angiogenic capacity. Doping of specific types of metallic elements (e.g., copper) into CaPs structure can create more potent angiogenic biomaterials. In addition, surface functionalization of CaPs bioceramics by proangiogenic growth factors and other chemicals was proven to be effective in improving neovascularization and accelerated wound healing. It is worth noting that CaPs can also be utilized for loading and delivery of proangiogenic cargoes (growth factors, phytochemicals, etc.) with the aim of accelerated tissue healing. Along with technological progress, the development of proangiogenic CaPs scaffolds by using three-dimensional (3D) printing provides great opportunities for researchers and scientists who work on tissue engineering and regenerative medicine. In this chapter, we have tried to present and criticize the angiogenic capacity of different types of CaPs bioceramics and draw a conclusion based on their usability for accelerating bone tissue healing based on the current knowledge.

Keywords

Calcium phosphates; Hydroxyapatite; Tricalcium phosphate (TCP); Angiogenesis; Neovascularization; Bone tissue engineering