The role of calcium phosphate surface structure in osteogenesis and the mechanisms involved

Review article

The role of calcium phosphate surface structure in osteogenesis and the mechanisms involved

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A B S T R A C T

Calcium phosphate (CaP) ceramics have been widely used for bone regeneration because of their ability to induce osteogenesis. Surface properties, including chemical composition and surface structure, are known to play a crucial role in osteoconduction and osteoinduction. This review systematically analyzes the effects of surface properties, in particular the surface structure, of CaP scaffolds on cell behavior and new bone formation. We also summarize the possible signaling pathways involved in the osteogenic differentiation of bone-related cells when cultured on surfaces with various structures *in vitro*. The significant immune response initiated by surface structure involved in osteogenic differentiation of cells is also discussed in this review. Taken together, the new biological principle for advanced biomaterials is not only to directly stimulate osteogenic differentiation of bone-related cells but also to modulate the immune response *in vivo*. Although the reaction mechanism responsible for bone formation induced by CaP surface structure is not clear yet, the insights on surface structure-mediated osteogenic differentiation and osteoimmunomodulation could aid the optimization of CaP-based biomaterials for bone regeneration.

Statement of Significance

CaP ceramics have similar inorganic composition with natural bone, which have been widely used for bone tissue scaffolds. CaP themselves are not osteoinductive; however, osteoinductive properties could be introduced to CaP materials by surface engineering. This paper systematically summarizes the effects of surface properties, especially surface structure, of CaP scaffolds on bone formation. Additionally, increasing evidence has proved that the bone healing process is not only affected by the osteogenic differentiation of bone-related cells, but also relevant to the the cooperation of immune system. Thus, we further review the possible signaling pathways involved in the osteogenic differentiation and immune response of cells cultured on scaffold surface. These insights into surface structure-mediated osteogenic differentiation and osteoimmunomodulated-based strategy could aid the optimization of CaP-based biomaterials.

1. Introduction

1.1. Bone and its healing

As the main component of the skeletal system, bone plays an important role in the protection, support, and motion of the body. Bone is a mineralized matrix that consists of 65% calcium phosphate, with the remaining mass comprising organic components and water. The organic components contain predominantly type I

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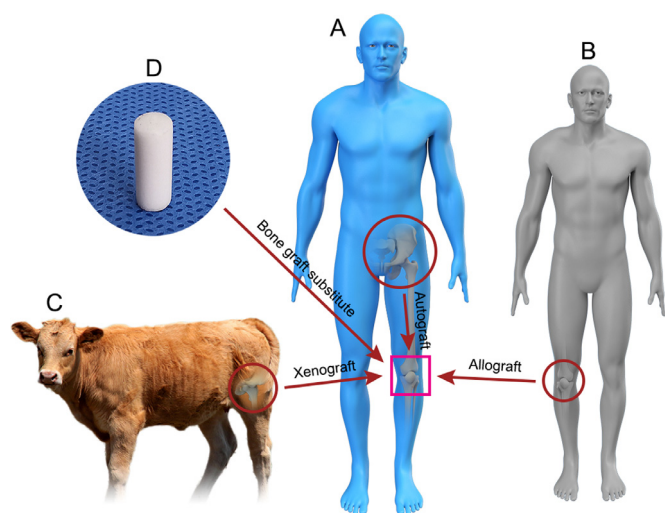[1] Both authors contributed equally to this work.

Fig. 1. Different types of bone grafts. (A) Autograft: The surgeon harvests bone from another site of the patient's skeleton. (B, C) Allograft and xenograft: The bone graft is obtained from a human donor or animals. (D) Synthetic bone graft substitute: There are different origins for synthetic grafts.

collagen and small fractions of important non-collagenous proteins (e.g. proteoglycans, osteopontin, osteonectin and osteocalcin) [1]. Bone hosts a series of cell, such as osteoblasts, osteocytes and osteoclasts. Osteoblasts synthesize bone matrix by secreting various extracellular matrix (ECM) proteins and participate in the mineralization process. While producing a mineralized bony matrix, osteoblasts are gradually entrapped in it, eventually becoming osteocytes. Being the most abundant cell type in the bone, osteocytes maintain the physiological functions of the skeleton by sensing mechanical strains and bone damage. Osteoclasts are located at the bone surface and resorb bone tissue by dissolving the calcium phosphate crystals and decomposing the organic matrix [2]. The bone is also a highly vascularized tissue capable of self-healing and remodeling [3]. The process of bone healing involves a cascade of biological events that are subdivided into four overlapping stages. Inflammation starts shortly after fracture, driven by special cytokines, angiogenic growth factors and osteogenic factors. Subsequently, bone healing involves soft callus formation followed by its maturation into a hard callus, leading to remodeling and final restoration of the geometry and function of the damaged bone. The mechanism underlying the healing process requires the regulation of chemotaxis, proliferation, differentiation, ECM synthesis, formation and remodeling of the newly formed bone at the fracture site.

1.2. Requirements of synthetic bone grafts

However, the bone self-healing process can be achieved only in small bone defects (i.e. non-critical-sized bone defects). For larger defects, grafting materials are frequently required to assist healing. The classical definition of a critical-sized bone defect is "the smallest intraosseous defect in a particular bone and species of animal that does not heal over the lifetime of the animal" [4]. For the most species, a length exceeding 1.5 times the diameter of the defect bone can be considered as the minimum critical size [5,6]. Natural (i.e., autografts, allografts and xenografts) and synthetic bone grafts are commonly used in the repair of these large bone defects (Fig. 1). Autografts are the gold standard due to their non-immunogenicity, osteoconductivity and osteoinductivity [7]. However, autografting requires additional surgical procedure and may cause donor-site pain and morbidity, thus limiting their use. Allografts and xenografts are possible alternatives to autograft, but they are ethically undesirable and may induce immunological

rejection [8]. Therefore, it is imperative to develop synthetic bone-grafting materials.

Synthetic bone grafts are available in different forms with various chemistries including polymers, metals, ceramics and their combinations. Among the possible synthetic materials, calcium phosphate (CaP) ceramics have been suggested as promising candidates for their chemical similarity to the human bone. Hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and their biphasic calcium phosphate composites (BCP) are the most investigated ceramics for bone regeneration. It is generally accepted that CaP ceramics are biocompatible and bioactive, support bone formation on their surface and form chemical bonds to the newly formed bone [9]. It is noteworthy that to repair critical sized bone defects, both conductive and inductive bone formation processes are necessary. Osteoconductivity is the ability of the material to allow unhindered bone ingrowth, while oseoinductivity is the ability of the material to trigger and support new bone formation in non-osseous sites in the absence of osteogenic factors [10,11]. Osteoconduction involves the recruitment and migration of bone-forming cells into the defect site, while osteoinduction triggers their differentiation into osteogenic cells and new bone formation [12]. Autografts are both osteoconductive and osteoinductive, while synthetics are generally only osteoconductive. However, several CaP ceramics were reported to induce bone formation at non-osseous sites without the addition of growth factors and/or cells in the past decades [13–15]. It was subsequently discovered that this osteoinductivity was correlated to the surface properties of CaP ceramics [16,17]. The differences in surface properties of CaP can influence the protein adsorption, consequently affecting the behavior of bone-related cells via the cell-extracellular matrix interactions and thus modulating the osteoinductive ability of bone grafts [18,19]. Therefore, understanding what roles the surface properties play in regulating cell behavior is critical for the design of osteoinductive CaP ceramics. This review focuses on the role of surface properties (including the surface chemistry and structure) of CaP ceramics in osteoconductivity and osteoinductivity both *in vitro* and *in vivo*. In addition, the importance of surface structure in osteogenesis is described, including the initiation of osteogenic differentiation of MSCs and immune response.

2. Material factors relevant to osteoinductive CaP ceramics

It has been reported that the macropore structure, the micropore structure and the chemistry are the three key factors of osteoinductive CaP ceramics. This section provides an analysis of how macropore/micropore structures and chemical composition of CaP scaffolds affect their osteoinductivity (Fig. 2).

2.1. Macropore structures

To be osteoinductive, CaP ceramics should have 3D macroporous structure. CaP-induced osteoinduction is rarely observed on flat surfaces. Scaffolds with well interconnected macropores ranging 300–500 μm are recommended, partly because this geometry ensures nutrients and metabolic waste transport, vascular ingrowth and direct osteogenesis [20,21]. Although many studies confirmed the important effects of pore size and porosity on cell growth and tissue formation, relatively few have focused on the role of the surface characteristics of the macropores in tissue growth [22,23]. Wang et al. suggested that the macroporous geometry of HA scaffolds plays a critical role in the vascularization and osteoinduction processes [23]. In this study, two categories of HA scaffolds with complementary macrostructures were prepared by spherulite-accumulating and porogen-leaching methods and implanted in canine muscles. Histological examination showed that, one month after implantation, the blood vessel density was greater in the
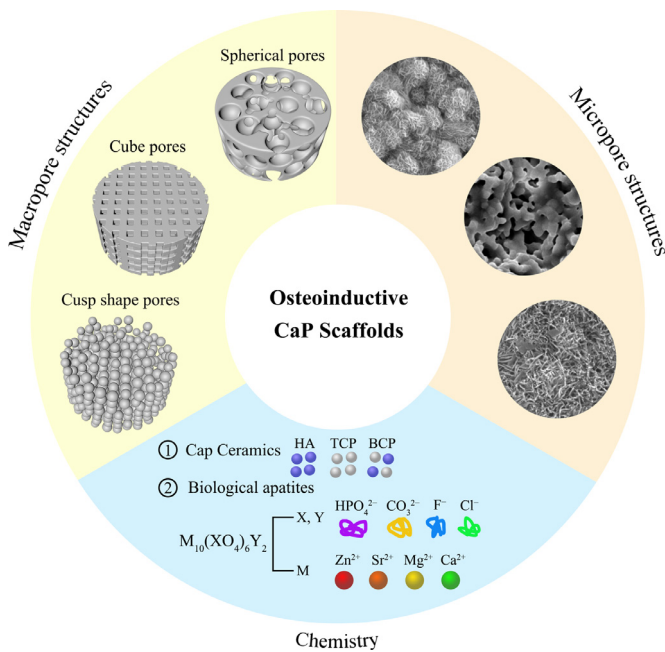
Fig. 2. Material characteristics (including macropore/micropore structure and chemical composition) relevant to osteoinduction of CaP ceramics.

porogen-leaching scaffolds than in the spherulite-accumulating ones. Further, more bone formation was observed in the latter category 3 and 6 months after implantation [23]. It was suggested that the macropores with convex surfaces (i.e., the spherulite-accumulating scaffolds) were likely more beneficial for liquid flowing and cell migration than those with concave surfaces (i.e., porogen-leaching scaffolds). In contrast, Ripamonti et al. [24] reported that bone formation in the rectus abdominis of baboon always started on the concave surfaces and never on the convex surfaces of HA discs. This difference may be partly attributed to the different HA materials (i.e., porous scaffolds vs. discs) and/or to the different animal models used. In addition, Rumpler et al. [25] found that the tissue growth rate *in vitro* was strongly affected by the channel geometry in HA plates. The new tissue started to form at the corners of the polygonal channels, regardless of the original shape. Tissue grew fastest on the surface of the round channels, followed by the triangular, the squared and then the hexagonal channels. These studies indicate that the tissue growth is affected by the local surface curvature of the macropores. In fact, some studies proposed a curvature-controlled tissue growth for predicting the geometrical pattern of tissue growth [22,26].

During bone remodeling, osteoclasts decompose the bone matrix and leave on its surface irregular pits or trails (e.g. grooves, semi-circular), which are later filled with osteoid by osteoblasts [27]. Despite this continuous local geometrical alteration, the mean surface curvature of the trabecular bone remains strictly controlled [28]. To understand the mechanism underlying this curvature-controlled tissue growth, Bidan et al. prepared circular pores and semi-circular channels (radius: 0.5 mm), mimicking osteons and osteoclastic resorption pits in HA scaffolds [22]. In the circular pores, the tissue grew in a concentric way and increased the average surface curvature, while the semi-circular channel tended to be flattened out by filling layer by layer. Faster growth occurred in circular pores in comparison to semi-circular surfaces, resembling a similar phenomenon in trabecular and cortical bone remodeling [29]. The authors [22] proposed that this curvature-controlled growth could be derived from the assembly of tensile elements on a curved substrate. The tensile elements

indicated cells anchored on distant points of the curved surface, thus creating an actin chord by generating tension between the adhesion sites. In this respect, the surface curvature of macropores ranging 300–500 μm may provide optimum tension on the cell's mechanoreceptors, as surface tension has been confirmed to be a determining factor affecting cell adhesion and tissue growth [30]. All these results imply that cell behavior and bone tissue formation may be highly dependent on the geometric properties of the scaffold's macropores. Deeper insights into this association may help understanding bone remodeling and improve the design of pore geometries for bone implants.

2.2. Micropore structures

Although macropore structure plays a key role in osteogenesis, micropores (<50 μm) on the CaP surface have also strong impact. An increasing number of studies shows that micropores improve bone in-growth into the macropores but also create additional space for bone ingrowth [31,32]. Micropore presence in CaP ceramics results in larger surface area, thus increasing protein adsorption, ion exchange and mineralized tissue formation [33]. Recent studies [34,35] reported that micropore-induced capillarity accelerates bone regeneration *in vivo* through the enhancement of the homogeneity of bone distribution in scaffold macropores. The significance of micropores in CaP ceramics was also highlighted in studies by Yamasaki and Yuan et al., where HA ceramics with micropores were observed to be osteoinductive after subcutaneous [36] and intramuscular [32,37] implantations in dogs, while no bone tissue was observed in those HA ceramics without micropores. Bohner et al. [31] implanted β-TCP scaffolds into ovine bone defects and found that cell/collagen-rich mineralized tissue occurred only in the interconnected micropores greater than 1–10 μm, regardless of the macropore size. We also found [18] abundant bone formation in TCP ceramics with micropore size of 1.58± 0.65 μm, as opposed to bone formation in those with micropore size of 0.65±0.25 μm. Therefore, micropore size is an effective modulator of the bone forming ability of CaP ceramics.

Other studies suggested that the osteoinductivity of CaP ceramics was enhanced by increasing the microporosity within appropriate ranges [13,38]. For instance, BCP ceramics having 17% microporosity induced bone formation in goats muscles, whiles no bone formation was observed in those having 3% microporosity [38]. In contrast, Lapczyna et al. [39] implanted TCP scaffolds with microporosity from 10% to 25% in ovine bone defect, but observed no microporosity-related differences in bone tissue formation. They only observed an increase in the resorption rate and soft tissue formation with the increasing microporosity. Apart from the effects on bone formation, microporosity also influences the incorporation and sustained release of proteins from the ceramic scaffolds [40,41]. When microporosity was <10%, more proteins were adsorbed onto CaP scaffolds with increasing microporosity [41]. Polak et al. [40] found that microporous BCP substrates adsorbed significantly more peptides having markedly higher release rate (vs. non-microporous ones). However, when porosity was increased from 50% to 60%, the total adsorption amount of peptides was not significantly different between the different microporous substrates. Results from these studies suggest that a rational design of microporosity characteristics in ceramic scaffolds plays important roles in osteoinductivity.

2.3. Chemistry composition

Besides macro/micro-porous structures, CaP chemistry is another key factor affecting the osteoinductive potential of scaffolds. The influence of the chemistry on the osteoinduction of CaP ceramics was seen in HA, TCP and BCP with various HA/TCP

ratios. When HA and BCP were both implanted into dogs, earlier and faster bone formation was observed in BCP than in HA. This indicated that BCP had a greater osteoinductivity than HA [42]. When HA, BCP and TCP were compared, the osteoinductive potential of CaP ceramics increased with the TCP content [13]. However, in some studies, osteoinduction occurred only in CaP ceramics having certain HA/TCP ratios, and the osteoinductive capacity did not enhance with the increase of TCP content [15,43]. These differences may be attributed to the intrinsic differences in the material properties such as stoichiometry, solubility, and microstructure. Among the various CaP types, HA is the most stable and least soluble, with a Ca/P ratio of 1.67 [44]. Although not highly soluble, its composition is closest to the natural bone mineral and its osteoconductivity can be tuned via chemical or structural modifications [45,46]. Compared with HA, TCP is more soluble under physiological conditions and has much greater osteoconductive capacity [42]. As it may be readily expected, the mixture of HA and TCP (i.e. BCP) possesses a controllable solubility and superior osteoconductivity depending upon the characteristics of the individual phases and HA/TCP mass ratio[15]. Taken together, the osteoinductive potential seems to follow a trend of BCP > TCP > HA. Although conflicting findings exist, they do suggest that the phase composition of CaP has a significant influence on the physicochemical properties and osteoinduction of scaffolds.

Trace elemental substitutions in CaP have also attracted remarkable interest in regulating cell behavior and bone regeneration. In fact, biological apatite is expressed as $M_{10}(XO_4)_6Y_2$, where M represents the partial substitution of Ca^{2+} by cations (e.g., Sr^{2+}, Cu^{2+}, Zn^{2+}, Na^+, K^+), X and Y represent anionic substitutions (e.g., HPO_4^{2-}, CO_3^{2-}, F^-, Cl^-) [47]. These substitutions have a fundamental influence on the crystal structure and dissolution behavior of biological apatite. For instance, carbonate substitution tends to decrease the apatite crystallinity but increases its solubility [48], Whereas F- substitution tends to form highly crystalline apatite with a decreased solubility [49]. Cations smaller than Ca^{2+} (e.g., Zn^{2+}, Mg^{2+}) tend to stabilize the crystal lattice of β-TCP, whereas larger ones (e.g. Sr^{2+}) tend to enter α-TCP [50–52]. More importantly, ion substitutions not only affect apatite crystallization and solubility, but also enhance its biological activity. Recent reviews have summarized the biological effects of metallic ions in CaP ceramics and their relevant active mechanisms in tissue regeneration [53–55]. It was suggested that the incorporation of ions such as Zn^{2+}, Sr^{2+}, Li^+ and Mn^{2+} into CaP ceramics could increase osteogenesis, and that the incorporation of Mg^{2+}, Cu^{2+} and Co^{2+} may enhance neovascularization. However, these studies predominantly focused on the substitution of a single ion in CaP ceramics. Recently, we simultaneously doped Cu^{2+} and Zn^{2+} into BCP scaffold [56] and found that with increasing dopant concentrations, the surface micromorphology of the scaffolds changed from smooth grains into rough microparticles and further into nanoflakes film. *In vitro* cell culture showed that the Cu/Zn co-doped BCP scaffolds exhibited a combined effect of angiogenic and osteogenic capacities [56]. Therefore, multiple substitutions seem to provide a promising approach toward the development of multifunctional CaP ceramics for bone regeneration.

Moreover, the chemical modification can significantly influence the surface wettability and surface charge, which in turn may affect cell adhesion and proliferation. Thian et al. [57] coated nanoHA (nHA), carbonate-substituted HA (nCHA) and Si-substituted HA (nSiHA) onto glass substrates. It was found that the nSiHA-coated surface showed a higher hydrophilicity and was more electronegatively charged than nHA and nCHA, which led to a significant increase in cell attachment and proliferation on nSiHA-coated surface. Similar results were observed by others [58,59], suggesting the positive effect of hydrophilicity on cell adhesion. In addition, clear evidence showed that nSiHA encouraged relevant cell

signaling to promote osteoblast differentiation, probably due to its more negative surface charge [57]. Nishizawa et al. [59] modified the surface of CaP ceramics with silane coupling reagents, and observed that a negative potential was effective in increasing cell adhesion. It was also shown that certain proteins were easily adsorbed onto CaP surface with negative surface charge, thereby triggering specific gene expression and protein synthesis toward osteogenesis [60]. Therefore, surface chemical modifications can fundamentally affect cell proliferation and differentiation by increasing the surface wettability and modulating the surface charge (negative charge preferred).

3. Biological functions of surface structure

3.1. BMSCs as a cell model for studying cell-surface interactions

Bone marrow stromal cells (BMSCs) have been isolated from bone marrow and are considered as the progenitor cells for bone formation. BMSCs can differentiate into multiple cell types, such as osteoblasts, adipocytes, chondrocytes and smooth muscle cells [61–63]. In a laboratory, the differentiation of BMSCs normally requires the presence of differentiation factors, which are supplied by the use of supplemented culture media that contain growth factors or cytokines (e.g. dexamethasone for osteogenic differentiation and insulin for adipogenic differentiation) [64,65]. As BMSCs represent a key example of adult stem cells and cell source for therapeutic applications, they are frequently used as cellular models for the investigation of the interactions between cells and materials during bone regeneration. In the following parts of this review, we mainly focus on BMSCs but discuss also other bone cell types such as osteoblast or osteoblast-like cells.

The *in vivo* environment of cells presents complex chemical and physical cues. In contrast, in an *in vitro* culture system cells are normally cultured on a designed surface immersed in a culture medium. The surface may present controlled geometrical features from macro- to nano-scales. It is becoming increasingly evident that MSCs are highly sensitive to their microenvironment and respond to various factors such as surface morphology, mechanical factors and surface roughness [66–68]. However, how surface features induce *de novo* bone formation *in vivo* remains unclear. This question is particularly challenging because varying surface features is frequently accompanied by changes in the surface chemistry, influencing on protein adsorption, ions release and ions precipitation. Several theories have been proposed to explain how physicochemical properties of CaP induce *de novo* bone formation *in vivo*. Firstly, Ca^{2+} and PO_4^{3-} ions are released from the CaP ceramics (soluble factors) [13,69]; secondly, carbonated apatite layers are deposited on the surface of CaP ceramics [70]; and thirdly, various growth factors (e.g. BMPs and VEGF) are adsorbed on the surface of CaP ceramics [13,14,71]. Notably, another theory suggests that the surface structure physically affects the response of cells and tissues, leading to osteoinduction [72]. This theory provides us with a new angle of looking at the function of surface microstructure.

3.2. Regulation of cell behaviors by surface structure

Surface structures from micro- to nano-scales (e.g., morphology, roughness) have been widely reported to affect the cell behaviors, including adhesion, spreading, cytoskeletal distribution and gene expression. Thus, the controlled fabrication of various surface structures is potentially valuable for many applications such as tissue engineering, implant design and basic cell biology. This section mainly focuses on the effects of the micro/nano-scaled size, morphology and roughness of surface structures on cell growth (Fig. 3). In this article, to be distinguished from the surface morphology,
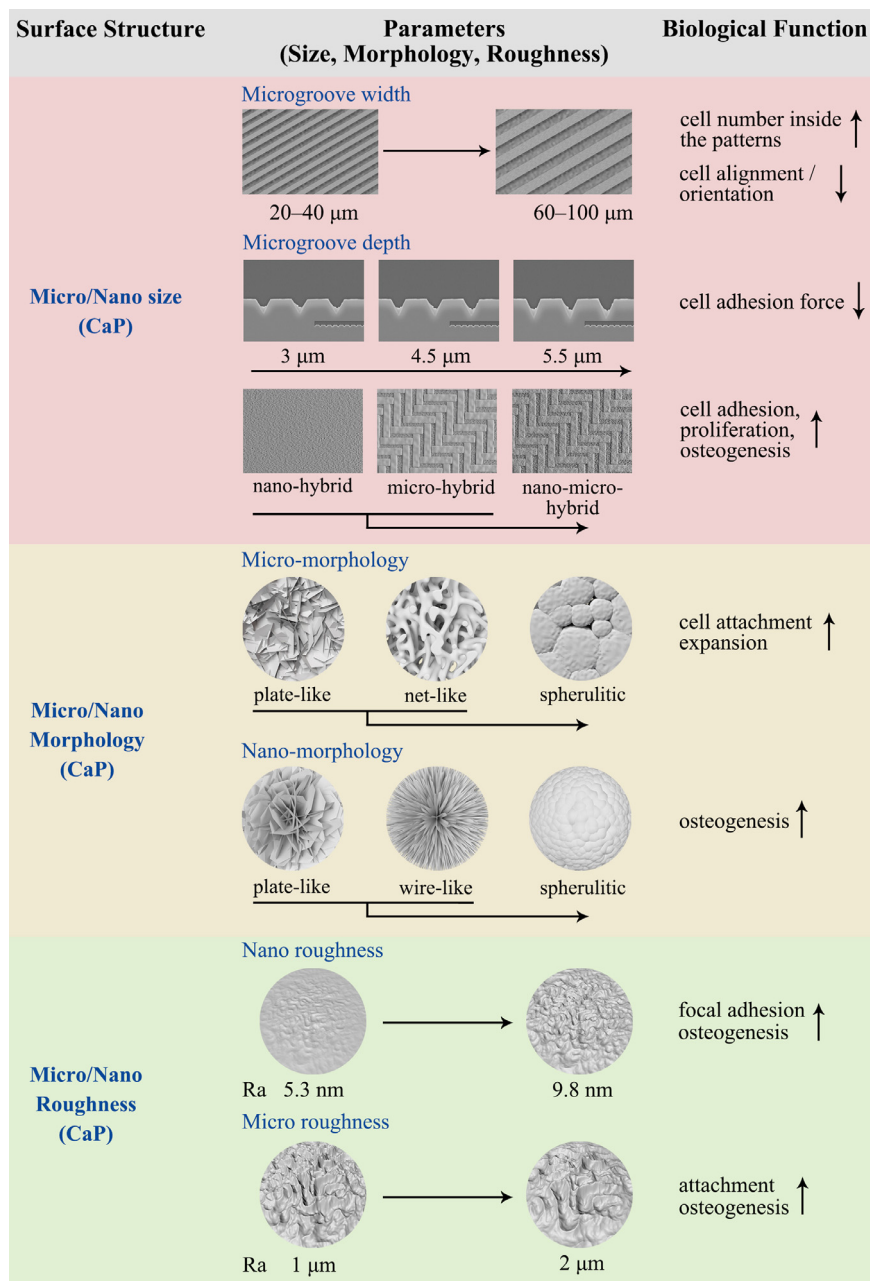
Fig. 3. Effects of surface structure, including structure size [79,8 1,88], morphology [84,85] and roughness [86,91], on cell behavior.

the surface roughness (quantitatively expressed by Ra) is defined as the arithmetic average of the absolute deviations of all points on a surface profile from its center line [73].

3.2.1. Micro-scale surface structure

The micro-scale structure could direct cell activities because most cells are micrometer-sized [74,75]. To investigate the effects of micro-scaled structural cues on cell responses, the fabrication of different microstructures on CaP surface via micro-patterns has attracted much interest [76–78]. Surface microstructures, including pillars, grooves and holes, on CaP surfaces were produced [76,79]. Different cellular responses to the micro-scale surface structure were also investigated regarding the cell shape, proliferation, differentiation and expression of proteins [76,79].

Among various microstructures (e.g. pillars, pores and grooves), microgrooves appeared to provide the most effective structural cues in guiding cell alignment [80,81]. In general, when the size

of microgrooves was within the micrometer range or was similar to the cell dimensions, they had strong effects in regulating the cell orientation and adhesion [79,81]. For instance, Holthaus et al. [81] found that, regardless of the microgroove depth (8~16 μm), widths of 60–100 μm showed more cells (35%–45%) inside the patterns compared with widths of 20–40 μm (16–25%). However, the number of aligned cells along the grooves with widths of 60–100 μm was significantly lower than on those having widths of 20–40 μm. This study suggested that microchannel widths similar to cell dimensions are probably more beneficial for guiding cell alignment, while the microchannel depth is a weak controlling parameter for cell orientation. However, Yang et al. [79] found that the adhesion force of an individual cell (osteoblast) to the substrate decreased with the increase of the depth of HA microgroove (ranging from 3 μm to 5.5 μm). Therefore, narrow ranges of width and depth of HA microgrooves may have a high impact on the single cell-guidance and adhesion. Notably, different cells are

sensitive to different micro-pattern sizes and surface topographic effects [82]. Lu and Leng [82] showed that the microgrooves with the width of 8 μm had strong contact guidance effects (cells alignment within the groove direction) on both myoblasts and osteoblasts, while those with the width of 24 μm strongly influenced myoblasts but not the osteoblasts. Therefore, cell type needs to be considered when designing the surface microstructure.

Apart from the size of micro-scaled structures, the shape of micro-scaled structure affects cell response as well. Akasaka et al. [76] compared the cell adhesion and proliferation on a series of shapes, including grooves, holes and pillars, having the same size (~2 μm). They found that osteoblast-like cells (Saos-2) spreaded well along the grooves, while filopodia were radially elongated on the pillars. Cells showed round morphology on the holes and on planar surfaces. Further, much more cells attached on the patterned apatite than on the planar apatite. Therefore, changes in size and shape of the microstructures can lead to significant changes in cell morphology, orientation, proliferation and adhesion force.

3.2.2. Nano-scale surface structure

Nanofabrication techniques have been increasingly developed and used to tailor nano-scaled surface structures of materials. More evidence is being collected on the importance of nano-scaled (<1 μm, or specifically ≤100 nm) structures in directing cell response. Cells may respond to nanostructures likely because the ECM is typically a network of nanosized collagen fibrils and because cellular receptors and filopodia are on the nanoscale. Therefore, nano-scaled structure may affect the type and conformation of proteins adsorbed, thereby mediating cell-material interactions.

Several studies have successfully prepared CaP surfaces with various nanostructures and analyzed the effects of nanostructures on the cell response [83,84]. For instance, Pang et al. [83] prepared HA coatings with various nanostructures (flake-like nanostructure vs. rod-like nanostructure) on a Ti substrate via hydrothermal-electrodeposition with the control of the electrolytes concentration. Compared to flake-like HA, rod-like nanoarray HA not only had better adsorption of fibronectin proteins, but also enhanced osteoblasts (MC3T3-E1) spreading, proliferation and osteogenic differentiation. Recently, we fabricated various nanostructures on the surface of HA scaffolds with the assistance of small organic molecules under hydrothermal condition [84]. Cell culture results showed that compared to plate-like or wire-like nanostructures, spherical nanostructures upregulated the expression of osteogenic genes (e.g., ALP, OPN) [84]. Moreover, for the investigation of the effects of CaP surface structure on cells, deposition of nanosized CaP coatings on a polymer substrate is also an effective method [85,86]. For instance, CaP coatings with various morphologies were deposited on bioresorbable polymers by immersion in simulated body fluids [85]. More stem cell attachment and expansion were observed on spherulitic micro-morphology than on plate-like or net-like micro-morphology [85]. These findings suggest that CaP with spherical morphologies may be more beneficial for cell attachment and osteogenic differentiation.

Regarding the hierarchical micro/nano-scaled structures of natural bone, it is reasonably presumed that hierarchical micro/nano-structure plays an important role in regulating cell behavior. A study performed by Xia et al. [87] showed that, compared with nanostructures (nanosheet or nanorod), micro-nano-hybrid (nanorod and microrod) structures on HA surface significantly enhanced BMSCs attachment, spreading and ECM deposition. Further, BMSCs cultured on micro-nano-hybrid surface had the best cell proliferation, the highest ALP activity as well as osteogenic gene expression (Runx2, BSP and OCN). Another study performed by Zhao et al. [88] showed that BMSCs responded differently to micro-, nano- and micro-nano-hybrid structures on HA surfaces. Micro-nano-hybrid structures (micropattern-nanorod-hybrid structure) showed higher cell adhesion, proliferation and ALP activity than a single-scale structure (including nanorod and micropattern). These studies seem to indicate that the combination of micro- and nano-structures has a more positive impact on the proliferation and osteogenic differentiation of BMSCs. Micro-nano-hybrid structures might have synergistic effects of the nano- and micro-structures on stem cell behavior [88]. Therefore, hierarchical micro-nano-hybrid structures could be a critical factor to be considered when designing optimal CaP materials for bone tissue engineering.

3.2.3. Surface roughness

In addition to the morphology and size of micro and nano structure, surface roughness of CaP ceramics also plays an important role in cell attachment, proliferation and differentiation [89,90]. For instance, Deligianni et al. [89] fabricated HA discs with three different roughness levels (Ra=0.733 ± 0.203, 2.856 ± 0.180 and 4.680 ± 0.433 μm) by polishing with SiC papers of p1200, p600 and p180, respectively. It was found that the surface roughness enhanced cell adhesion, detachment strength and proliferation of BMSCs, but did not affect ALP activity of BMSCs. In another study, when compared against a flat CaP surface sputter deposited on a glass substrate (Ra = 5.3 ± 0.2 nm), osteoblast-like cells cultured on a rough CaP surface on Ti interlayer (Ra =9.8 ± 0.5 nm) exhibited upregulated focal adhesion assembly, osteocalcin expression and ALP activity [91]. Costa et al. [86] deposited HA coatings with varying surface roughness on polycaprolactone (PCL) discs by changing the concentration of simulated body fluid and found that osteoblast attachment and expression of osteogenic markers (Col1a2, Alpl and Bglap) were enhanced on the rougher discs (Ra = ~2 μm) than on the smoother ones (Ra = ~1 μm). These studies showed that the surface roughness is important for guiding cell behaviors, and the rough surface seems more favorable for cell attachment and differentiation. However, it is also reported that rough surface caused cell death. Zan et al. [90] prepared CaP coatings on polyelectrolyte multilayers using a biomimetic *in situ* growing method, and observed that the roughness window suitable for BMSCs growth was between 18±1.2 and 187±7.3 nm, while further increasing the roughness led to cell death. More specifically, the most favorable roughness for osteogenesis of BMSCs was ~98±3.5 nm, which resulted in the highest calcium deposits and osteocalcin. Therefore, the effects of surface roughness on the cell behavior cannot be evaluated simply from the value of Ra (nano- or micrometer). Instead, it depends on subtle differences in surface roughness that can be sensed by cells. A balance in surface roughness is critical, and it is a key factor in designing a successful biomaterial implant for bone regeneration.

3.3. Regulation of cell behaviors by protein-surface interactions

After implantation, a biomaterial surface is immediately exposed to the body fluid and blood containing a large variety of proteins. These proteins rapidly adhere to the biomaterial surface. Therefore, protein-surface interaction plays a critical role in mediating cell response to the biomaterial. The migration and adhesion of bone-forming related cells on a biomaterial surface are mediated via integrins (e.g. fibronectin, vitronectin) [92]. After integrin-mediated adhesion, transmembrane receptors cluster to activate signaling cascades followed by cell proliferation and differentiation. Protein-surface interaction is not only dependent on the surface characteristics (e.g., chemistry, morphology, roughness), but it is also correlated with the adsorbed proteins on the CaP surface *in vivo*. Therefore, understanding and controlling the protein-surface interactions may allow tailoring the scaffold surfaces to enhance osteoinductivity.

First, the surface chemistry of CaP plays a key role in the adsorption of proteins. Compared with other biomaterials, CaP ceramics have a high adsorption capacity for serum proteins. Studies found that CaP surfaces can adsorb more bovine serum albumin than Ti surfaces [93]. It was theorized that protein adsorption on CaP occurs via electrostatic interactions involving at least two possible sites [94]: positively charged Ca sites (adsorbing acidic proteins) and negatively charged P sites (adsorbing basic proteins) [95]. Luo and Andrade reported that the adsorbing affinity of Ca sites was greater than that of the P sites [96]. Ohta et al. [60] studied the adsorption characteristics of different proteins on several CaPs, including β-TCP, $CaHPO_4 \cdot 2H_2O$ (DCPD), $CaHPO_4$ (DCPA), $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ (OCP) and HA. They found that the ratios of Ca/P sites were related to the electrostatic charges of different proteins, showing a tendency of: DCPD>OCP>HA≫DCPA≫ β-TCP. The difference in Ca/P sites ratios of the various CaP was mainly determined by the crystal structures, which exposed more Ca or P sites on the crystal surface. On the other hand, the protein adsorption on the various CaP surface matched the rank of surface zeta potentials of different CaPs [60]. In addition, compared to HA, BCP always had a higher adsorption capacity for fibrinogen, insulin or Collagen I [41]. It is well known that β-TCP has a higher solubility than HA, and its dissolution increases ionic strength of the solution, resulting in a stronger interaction between protein ionized amino acid residues and surface binding sites of BCP [97]; Therefore, the total amount and types of adsorbed proteins were determined by the surface chemical properties of CaP, including surface zeta potential, crystal structure and solubility.

Second, the protein-surface interaction is determined cooperatively by the surface chemistry and the surface structure, but their contributions are frequently difficult to differentiate. To isolate the effect of surface structure on protein adsorption, Santos et al. [98] used gold-sputtering to coat the surfaces of HA and β-TCP samples to mask the surface chemical effects on protein adsorption (without changing the original roughness). The adsorptions of albumin and fibronectin were both higher on the HA surface than on β-TCP, indicating a strong structural effect on protein adsorption. This was explained by the nanostructure of the HA samples, which induced more albumin and fibronectin adsorption than did β-TCP. Other findings indicated that surface roughness affects the adsorption kinetics of proteins only when it is within the protein size scale. When the roughness exceeds the size range of proteins, the surface appears smooth to proteins and, in this case, the surface chemistry dominates the protein adsorption kinetics [99,100]. Apart from roughness, surface curvature was reported to affect the activity of adsorbed proteins. Roach et al. found that [101] the native-like conformation of a globular protein (albumin) was stabilized by high surface curvature, while a rod-like protein (fibrinogen) was induced to lose the secondary structure by wrapping around surface curvature. Alternatively, the protein adsorption on the CaP surface may change the mineral nucleation rate and inhibit mineral crystal formation [102]. However, there is no universal agreement in categorizing a protein as a nucleator or inhibitor, which is dependent on the characterization system and protein concentrations.

Third, competitive and selective adsorption of different proteins onto surfaces needs to be characterized to understand *in vivo* protein adsorption. The diffusion chamber is widely used for this purpose as it allows ions and proteins to enter into it while excluding cells [103]. Zhu et al. [104] investigated the adsorption of serum proteins on BCP scaffolds with different surface structures using a diffusion chamber model. They found that scaffolds with high microporosity provided more binding sites for protein adsorption due to its higher specific surface area. Additionally, the total protein adsorption did not increase with the time but the specific transforming growth factor-β1 (TGF-β1) adsorption increased

significantly. This suggested that TGF-β1 had a stronger affinity to BCP and thus concentrated on its surface. As bone growth factors (e.g. TGF-β1) play key roles in the proliferation and differentiation of bone-forming related cells, their adsorption on CaP and subsequent effects on cell function may provide important insights into the mechanism underlying the osteoinduction of CaP ceramics.

4. The mechanism of surface structure in osteogenesis

4.1. Biological signals involved in osteogenic differentiation of MSCs

Although increasing evidence supports the hypothesis that surface structures are responsible for osteogenic differentiation of hBMSCs, the mechanisms remain unclear. It has been established that, during lineage commitment, cells undergo morphological changes [105,106]. Conversely, cell morphology has also been found to regulate cellular differentiation [107,108]. In this section, we review some prominent surface structure-induced biological signals implicated in cell adhesion, proliferation and differentiation.

Primary cilia are microtubule-based, hair-like sensory organelles located on most mammalian cells. These organelles sense mechanical and biochemical stimuli and translate these into intracellular signals to direct cell growth and tissue homeostasis [109,110]. *In vitro* experiments observed that MSCs cultured on polymer grooved structures (width: 12.5 μm) expressed elongated primary cilia via reducing actin organization [110]. In a study [72] we seeded BMSCs on TCP discs with micrometer- (TCP-B) and submicron meter-scale (TCP-S) surface structures, and found that the TCP-S increased the length of the primary cilia and ciliary recruitment of TGFβ associated with osteogenesis. Similar results were observed in the cilia-associated mechanisms of osteogenesis of adipose-derived stem cells (ASCs) [111]. After culture of ASCs in an osteogenic differentiation medium, their primary cilium structures became elongated, suggesting a relationship between cilium structure and phenotypic determination. Thus, surface structure control of osteogenesis via cilia modulation may provide a new biomaterial-based strategy for bone regeneration.

Some key molecules contribute significantly to integrin-mediated cell-ECM adhesion at the early stage, such as focal adhesion kinase and Src family kinases [112]. Recent studies revealed the effects of surface structures on the focal adhesion and downstream stem cell behaviors [113,114]. Bello et al. [115] observed an increased adhesion per cell area and focal adhesion length from osteogenic cells cultured on discs with nanoscale structure. Meanwhile, significantly enhanced gene expression for focal adhesion markers (integrins, paxillin and talin) were recorded in cells adhering to the nanostructured surface compared with polished surface. Another study showed that, when BMSCs were cultured on the micro/nano-structured HA surfaces, the extracellular signal-related kinases (ERK) and p38 mitogen-activated protein kinase (MAPK) signaling pathways were activated [87]. MAPK/ERK signaling pathways are known to control cell functions such as proliferation and differentiation [116,117]. When BMSCs were cultured on the micro/nano structured HA scaffolds, ERK and p38 MAPK signaling pathways became phosphorylated, especially for a micro/nano-hybrid structure [87]. When the signaling pathways were blocked by ERK and p38 MAPK inhibitors, the osteogenic differentiation of BMSCs was attenuated [87]. These results indicated that BMSCs might have sensed micro/nano-structured surfaces through focal adhesion formation and activated ERK and p38 signaling pathways, upregulating relevant genes and leading to proliferation and osteogenic differentiation. Additionally, other signaling events (e.g., TGF-β/BMP and Wnt signaling) related to proliferation and differentiation may also participate in the structure sensation of stem cells. Yang et al. [118] found that surfaces

with nanopits promoted the colocalization of integrins and BMP2 receptors, and upregulated the transcriptional activity of RUNX2 and SMAD1/5, thereby enhancing the osteogenic activity of stem cells. Further, HA ceramics with micro/nano-hybrid structures (composed of nanorods and micropatterns) firstly activated integrins followed by BMP2 signaling pathway and Cx43 expression, which enhanced MSCs adhesion, proliferation and osteogenic differentiation [88]. HA ceramics with a micro/nano-hybrid surface (the hybrid of nano-rods (diameter: 80–120 nm) and micro-rods (diameter: 1–4 μm) was also reported to enhance the gene expressions of low-density lipoprotein receptor-related protein 5 (LRP5) and β-catenin, two important genes related to the canonical Wnt signaling pathway [119]. The expression of osteogenic genes (e.g., ALP, OCN, RUNX2) was suppressed by adding canonical Wnt signaling inhibitor [119]. Moreover, recent studies demonstrated that the Yes-associated protein (YAP)/PDZ binding motif (TAZ) signaling pathway participated in the regulation of BMSCs osteogenic differentiation [120,121]. Yang et al. [120] found that HA discs with rougher surface (Ra=0.77~1.09 μm) increased cell attachment and promoted the levels of TAZ and YAZ proteins in BMSCs, resulting in osteogenic gene expression and mineralization. The authors proposed that the surface roughness regulated cell adhesion, spread and morphology for regulation of the cytoskeletal tension. This tension was transferred to nuclear transcription factors YAP/TAZ via the cytoskeleton, followed by binding to the osteogenic transcriptional factor RUNX2, which led to osteogenic differentiation [120]. Another study found that instead of a single signaling pathway, multiple pathways may be simultaneously involved in regulating mechanotransduction-induced osteogenesis. For instance, TGF-β/BMP, Wnt and MAPK signaling pathways were all found activated in BMSCs cultured on random nanofibers [122]. Therefore, analogous to biochemically driven cascades, and in response to surface structures, multiple signaling pathways are involved in the adhesion, morphology change and osteogenic differentiation of BMSCs (Fig. 4). Further, a network of signaling pathways may be responsible for the mechanism underlying surface structure-mediated osteogenesis, rather than a single signaling pathway.

Remarkably, osteoblast precursor cells cultured on three-dimensional scaffolds were found to experience greater stress stimulus than those on two-dimensional surfaces [123]. This explained that the unique curvatures of three-dimensional scaffold, analogous to the trabecular bone, could provide substantial micro/nano-scaled surface characteristics that are not present on two-dimensional surfaces. These characteristics significantly activate the stress mediators p38 and c-Jun N-terminal kinase (JNK), but not ERK1/2, suggesting a cell ability to manage high stress signals in response to surface structure of three-dimensional scaffolds [123]. Therefore, in future studies, the design mimicking the curvature structure of trabecular bone will help to understand mechanism of cell-material interaction in bone regeneration.

4.2. Mechanism of immune responses involved in osteogenic differentiation of MSCs

Bone defect healing is initiated by inflammation, followed by angiogenesis, bone morphogenesis and bone remodeling. Immediately after implantation, proteins in the blood and tissue fluids (e.g. vitronectin, fibrinogen and fibronectin) reach and adsorb onto the biomaterial surface, resulting in the activation of the coagulation cascade and complement system. Then, an acute inflammatory response is initiated and amplified, featuring the recruitment and activation of polymorphonuclear leukocytes (PMNs), neutrophils and macrophages. These immune cells release cytokines and regulate inflammation, thus inducing or inhibiting bone formation [124,125]. It has been shown that monocytes/macrophages cultured on submicron-structured CaP ceramics without RANKL

also induce osteogenic differentiation, indicating that inflammatory cells could respond to surface structure and produce osteogenic cytokines for osteoinduction [126]. The degradation products of BCP ceramics have been found to stimulate macrophages to secrete immunoregulatory cytokines and growth factors, which recruit MSCs and promote their differentiation into osteoblasts [127,128]. Thus, osteogenesis is accomplished not only by bone-related cells from the musculoskeletal system, but also by the collaboration of multiple physiological systems. Accordingly, Chen et al. [129] proposed the concept of "osteoimmunomodulation", which emphasizes the key role of the immune response in biomaterial-mediated osteogenesis *in vivo*. Thus, surface modifications of biomaterials may create a favorable microenvironment for the immune response to improve osteogenesis and avoid the formation of a fibrous encapsulation. Chen et al. [130] studied the effects of nanoporous anodic alumina with different pore size on the macrophage response and osteogenic differentiation of BMSCs. They found that the nanopore structure and pore size affected macrophages adhesion (including spreading and shape), activated autophagy pathway components and resulted in an anti-inflammatory reaction and release of osteogenic factors. Subsequently, the osteogenic pathways (BMP and Wnt) were enhanced by the nanopore-induced inflammatory environments (Fig. 5) [130]. Thus, the effects of immune cells on surface structured-mediated osteogenesis provide cues for designing advanced bone biomaterials with immunotherapeutic functions. Another study reported that instead of nanostructured surface, microstructured surface promoted the activation of M1 and M2 state of macrophages by inducing the expression of inflammatory cytokine (IL-1 and IL-6) and genes (e.g. IL1R and S100A9 for M1, STAB1 and CD163 for M2) [131]. M1-polarized macrophages are pro-inflammatory, whereas M2 phenotype macrophages are pro-healing (i.e. promoting angiogenesis and matrix remodeling) [132]. However, surface grooves did not affect inflammatory activation but drove macrophages toward a pro-healing phenotype in the report of Luu et al. [133]. When poly PCL fibers were implanted into rats, it was found that compared to solid PCL, the fibrous structure of PCL fibers enhanced MSCs recruitment via promoting macrophage recruitment, M1-to-M2 transition and SDF-1 secretion that subsequently led to the recruitment of MSCs [134]. In addition, it was also reported that during the bone remodeling stage the cells responsible for bone resorption also responded to surface structures [135]. Human CD14+ monocytes could be differentiated into functional osteoclasts when cultured on submicron-structured CaP ceramic (TCP-S), but not on micro-structured CaP ceramic (TCP-B) [136]. Jimbo et al. [137] investigated gene expressions in tissues around nanostructured CaP-coated implants and found that the ALP expression was enhanced, while tumor necrosis factor-α expression was reduced. This suggested that the inflammatory responses were suppressed and osteogenic activity enhanced around the CaP-coated surface. Moreover, adenosine triphosphatase, an osteoclast marker, was significantly higher in cells cultured on the CaP-coated Ti implants, showing a gradual resorption of the CaP coating [137]. This study provides evidence that the effects of surface structure are significant during the osseointegration cascade, not only on osteogenesis, but also on both inflammatory reactions and osteoclastogenesis.

Taken together, although limited studies have verified its effectiveness, it is hypothesized that surface structured-mediated osteoimmunomodulation may provide a promising strategy in regulating the osteogenic behavior of bone-related cells and determining the *in vivo* performance of CaP implants. More importantly, as the immunomodulatory response is triggered by protein adsorption and subsequent cell adhesion and growth, future biomaterial design should not only focus on the osteogenic differentiation of bone-related cells (e.g., vascular endothelial cells, MSCs, osteoblasts, osteocytes and osteoclasts) but also aim to
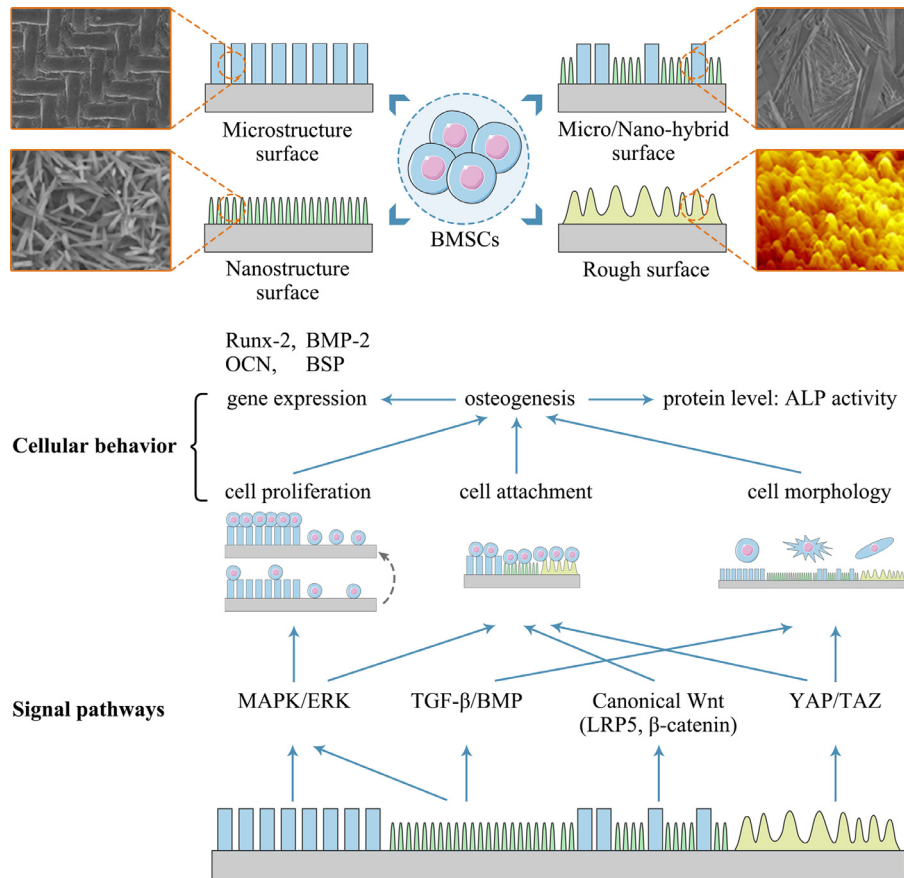
Fig. 4. Various signaling pathways involved in osteogenic differentiation of BMSCs responding to various surface structures.
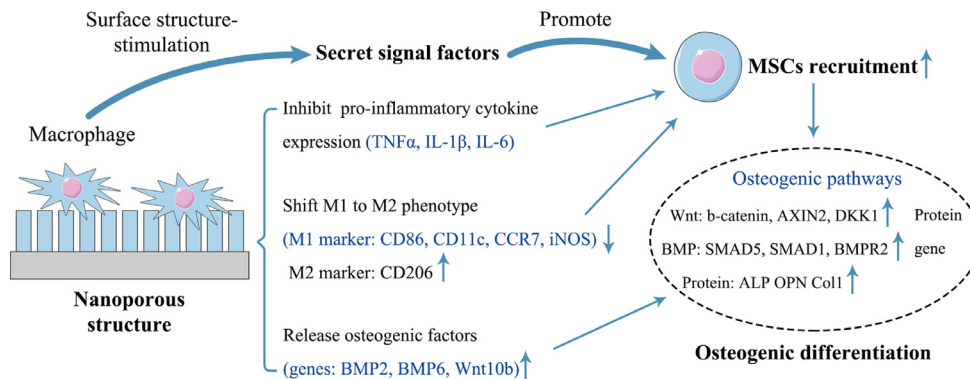

Fig. 5. Schematic illustration of the involvement of signaling factors secreted by macrophages cultured on the nanoporous structure and their effects on the osteogenic differentiation of BMSCs.

enhance the selective adsorption of desired proteins and modulate subsequent cell phenotype, thus generating a favorable immune environment for bone regeneration.

5. Summaries and outlook

Calcium phosphate ceramics have a similar inorganic composition with the natural bone and can serve as scaffolds in bone repair. However, CaP ceramics in solid or non-porous forms are not osteoinductive. Osteoinductivity can be imparted to CaP ceramics by surface engineering, such as the creation of appropriate macro/microscopic pores, surface morphology and roughness. An appropriate surface structure can optimize the ability to concentrate specific proteins or cytokines, to recruit immune cells and

osteoprogenitor cells and to subsequently affect cells adhesion, proliferation and differentiation into osteoblasts, thereby inducing bone formation.

Future studies may aim at a better understanding of the molecular mechanism of stem cells response to surface structures and eventually undergoing osteogenic differentiation. To achieve this, selective adsorption kinetics of various proteins on the scaffolds' surface should be thoroughly investigated to generate tailor-made surfaces that provide ideal microenvironments for the enhancement of cell adhesion, morphology, proliferation and differentiation. Moreover, published studies have demonstrated that the surface modification can regulate the response of immune cells, thus generate the osteo-immune environment for bone regeneration. However, compared to surface-mediated osteogenic

differentiation of stem cell, investigations into the interaction between the surface structure and immune cells are still in its infancy. In addition, how surface chemistry and structure modify the immune cells response, and how their response affects new bone formation, is not yet well understood. Moreover, material surface-mediated osteoclastogenesis has not yet been fully elaborated. Clarification of these topics may contribute to the design of osteoimmunomodulating CaP scaffolds able to control cell behaviors and induce new bone formation. Careful design of studies to investigate the role of inflammation and osteoclastogenesis in osteoinduction is expected to generate new insights on the biological mechanism of material-induced bone formation. Finally, exploring novel techniques to characterize biological processes after *in vivo* implantation of osteoinductive CaP can provide effective tools for the research of tissue-inducing biomaterials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

References

[1] M.J. Olszta, X. Cheng, S.J. Sang, R. Kumar, Y.Y. Kim, M.J. Kaufman, E.P. Douglas, L.B. Gower, Bone structure and formation: a new perspective, Mater. Sci. Eng. R 58 (2007) 77–116.

[2] T. Bellido, Osteocyte-driven bone remodeling, Calcif. Tissue Int. 94 (2014) 25–34.

[3] J. Buckwalter, M. Glimcher, R. Cooper, R.R. Recker, Bone biology. II: Formation, form, modeling, remodeling, and regulation of cell function, Instr. Course Lect. 45 (1996) 387–399.

[4] J.P. Schmitz, J.O. Hollinger, The critical size defect as an experimental model for craniomandibulofacial nonunions, Clin. Orthop. Relat. Res. 205 (1986) 299-308.

[5] B.H. Clough, M.R. McCarley, C.A. Gregory, A simple critical-sized femoral defect model in mice, J. Vis. Exp. 97 (2015) e52368.

[6] Z. Gugala, R.W. Lindsey, S. Gogolewski, New approaches in the treatment of critical-size segmental defects in long bones, Macromol. Symp. 253 (2007) 147–161.

[7] C. Faldini, F. Traina, F. Perna, R. Borghi, M. Nanni, M. Chehrassan, Surgical treatment of aseptic forearm nonunion with plate and opposite bone graft strut. Autograft or allograft? Int. Orthop. 39 (2015) 1–7.

[8] V. Campana, G. Milano, E. Pagano, M. Barba, C. Cicione, G. Salonna, W. Lattanzi, G. Logroscino, Bone substitutes in orthopaedic surgery: From basic science to clinical practice, J. Mater. Sci. Mater. Med. 25 (2014) 2445–2461.

[9] R.J. Miron, A. Sculean, Y. Shuang, D.D. Bosshardt, R. Gruber, D. Buser, F. Chandad, Y. Zhang, Osteoinductive potential of a novel biphasic calcium phosphate bone graft in comparison with autografts, xenografts, and DFDBA, Clin. Oral. Implants Res. 27 (2016) 668–675.

[10] X.D. Zhang, P. Zou, C. Wu, Y. Qu, J.G. Zhang, A study of porous block HA ceramics and its osteogenesis, in: Bioceramics and the Human Body, Elsevier, Amsterdam, 1991, pp. 408–415.

[11] U. Ripamonti, Bone induction in nonhuman primates: An experimental study on the baboon, Clin. Orthop. Relat. Res. 269 (1991) 284–294.

[12] J.E. Davies, M.M. Hosseini, Histodynamics of endosseous wound healing, Bone Eng. 1 (2000) 1–14.

[13] H. Yuan, H. Fernandes, P. Habibovic, J.D. Boer, A.M.C. Barradas, A.D. Ruiter, W.R. Walsh, C.A.V. Blitterswijk, J.D.D. Bruijn, Osteoinductive ceramics as a synthetic alternative to autologous bone grafting, PNAS 107 (2010) 13614–13619.

[14] H. Pamela, Y. Huipin, C.M. Valk, M.G. Van Der, C.A. Blitterswijk, Van, D.G. Klaas, 3D microenvironment as essential element for osteoinduction by biomaterials, Biomaterials 26 (2005) 3565–3575.

[15] Z. Tang, Y. Tan, Y. Ni, J. Wang, X. Zhu, Y. Fan, X. Chen, X. Yang, X.D. Zhang, Comparison of ectopic bone formation process induced by four calcium phosphate ceramics in mice, Mater. Sci. Eng. C 70 (2017) 1000–1010.

[16] N.L. Davison, J. Su, H. Yuan, V.D.B. Jj, J.D. de Bruijn, G.F. Barrère-De, Influence of surface microstructure and chemistry on osteoinduction and osteoclastogenesis by biphasic calcium phosphate discs, Eur. Cell Mater. 29 (2015) 314–329.

[17] Y. Zhu, K. Zhang, R. Zhao, X. Ye, X. Chen, Z. Xiao, X. Yang, X. Zhu, K. Zhang, Y.J. Fan, X.D. Zhang, Bone regeneration with micro/nano hybrid-structured biphasic calcium phosphate bioceramics at segmental bone defect and the induced immunoregulation of MSCs, Biomaterials 147 (2017) 133–144.

[18] J. Zhang, X. Luo, D. Barbieri, A.M. Barradas, J.D. de Bruijn, C.A. Van Blitterswijk, H. Yuan, The size of surface microstructures as an osteogenic factor in calcium phosphate ceramics, Acta Biomater. 10 (2014) 3254–3263.

[19] J. Wang, H. Zhang, X. Zhu, H. Fan, Y. Fan, X.D. Zhang, Dynamic competitive adsorption of bone-related proteins on calcium phosphate ceramic particles with different phase composition and microstructure, J. Biomed. Mater. Res. Part B Appl. Biomater. 101 (2013) 1069–1077.

[20] C.M. Murphy, M.G. Haugh, F.J. O'Brien, The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering, Biomaterials 31 (2010) 461–466.

[21] Y. Kuboki, Q. Jin, H. Takita, Geometry of carriers controlling phenotypic expression in BMP-induced osteogenesis and chondrogenesis, J. Bone Jt. Surg. 83 (2001) S105–S115.

[22] C.M. Bidan, K.P. Kommareddy, R. Monika, K. Philip, Y.J.M. Bréchet, F. Peter, J.W.C. Dunlop, How linear tension converts to curvature: geometric control of bone tissue growth, PLoS One 7 (2012) e36336.

[23] H. Wang, W. Zhi, X. Lu, X. Li, K. Duan, R. Duan, Y. Mu, J. Weng, Comparative studies on ectopic bone formation in porous hydroxyapatite scaffolds with complementary pore structures, Acta Biomater. 9 (2013) 8413–8421.

[24] U. Ripamonti, J. Crooks, A.J.S. Kirkbrideb, Sintered porous hydroxyapatites with intrinsic osteoinductive activity: geometric induction of bone formation, S. Afr. J. Sci. 95 (1999) 335–343.

[25] M. Rumpler, A. Woesz, J.W. Dunlop, J.T. van Dongen, P. Fratzl, The effect of geometry on three-dimensional tissue growth, J. R. Soc. Interface 5 (2008) 1173–1180.

[26] U. Ripamonti, Biomimetism, biomimetic matrices and the induction of bone formation, J. Cell. Mol. Med. 13 (2009) 2953–2972.

[27] A.M. Parfitt, Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone, J. Cell. Biochem. 55 (1994) 273–286.

[28] H. Jinnai, H. Watashiba, T. Kajihara, Y. Nishikawa, M. Takahashi, M. Ito, Surface curvatures of trabecular bone microarchitecture, Bone 30 (2002) 191–194.

[29] M.O. Agerbæk, E.F. Eriksen, J. Kragstrup, L. Mosekilde, F.J.B. Melsen, A reconstruction of the remodelling cycle in normal human cortical iliac bone, Bone Miner. 12 (1991) 101–112.

[30] T. Lecuit, P. Lenne, Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis, Nat. Rev. Mol. Cell Biol. 8 (2007) 633–644.

[31] M. Bohner, G. Baroud, A. Bernstein, N. Döbelin, L. Galea, B. Hesse, R. Heuberger, S. Meille, P. Michel, B. Von Rechenberg, Characterization and distribution of mechanically competent mineralized tissue in micropores of β-tricalcium phosphate bone substitutes, Mater. Today 20 (2017) 106–115.

[32] R. Duan, D. Barbieri, X. Luo, J. Weng, C. Bao, J.D. De Bruijn, H. Yuan, Variation of the bone forming ability with the physicochemical properties of calcium phosphate bone substitutes, Biomater. Sci. 6 (2018) 136–145.

[33] L.E. Rustom, M.J. Poellmann, A.J.W. Johnson, Mineralization in micropores of calcium phosphate scaffolds, Acta Biomater. 83 (2019) 435–455.

[34] S. Polak, L. Rustom, G. Genin, M. Talcott, A.W. Johnson, A mechanism for effective cell-seeding in rigid, microporous substrates, Acta Biomater. 9 (2013) 7977–7986.

[35] L.E. Rustom, T. Boudou, S. Lou, I. Pignot-Paintrand, B.W. Nemke, Y. Lu, M.D. Markel, C. Picart, A.J.W. Johnson, Micropore-induced capillary enhances bone distribution in vivo in biphasic calcium phosphate scaffolds, Acta Biomater. 44 (2016) 144–154.

[36] H. Yamasaki, H. Sakai, Osteogenic response to porous hydroxyapatite ceramics under the skin of dogs, Biomaterials 13 (1992) 308–312.

[37] H. Yuan, K. Kurashina, J.D. de Bruijn, Y. Li, K. De Groot, X.D. Zhang, A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics, Biomaterials 20 (1999) 1799–1806.

[38] P. Habibovic, H. Yuan, M. van den Doel, T.M. Sees, C.A. van Blitterswijk, K.J. Joor, K. de Groot, Relevance of osteoinductive biomaterials in critical-sized orthotopic defect, J. Orthop. Res. 24 (2006) 867–876.

[39] H. Lapczyna, L. Galea, S. Wüst, M. Bohner, S. Jerban, A. Sweedy, N. Doebelin, N. Van Garderen, S. Hofmann, G. Baroud, Effect of grain size and microporosity on the in vivo behaviour of β-tricalcium phosphate scaffolds, Eur. Cells Mater. 28 (2014) 299–319.

[40] S.J. Polak, J.S. Lee, W.L. Murphy, S. Tadier, L. Grémillard, I.V. Lightcap, A.J.W. Johnson, Microstructural control of modular peptide release from microporous biphasic calcium phosphate, Mater. Sci. Eng. C 72 (2017) 268–277.

[41] X. Zhu, H. Zhang, H. Fan, W. Li, X.D. Zhang, Effect of phase composition and microstructure of calcium phosphate ceramic particles on protein adsorption, Acta Biomater. 6 (2010) 1536–1541.

[42] H. Yuan, C. Van Blitterswijk, K. De Groot, J.D. de Bruijn, A comparison of bone formation in biphasic calcium phosphate (BCP) and hydroxyapatite (HA) implanted in muscle and bone of dogs at different time periods, J. Biomed. Mater. Res. Part A 78 (2006) 139–147.

[43] K. Kurashina, H. Kurita, Q. Wu, A. Ohtsuka, H. Kobayashi, Ectopic osteogenesis with biphasic ceramics of hydroxyapatite and tricalcium phosphate in rabbits, Biomaterials 23 (2002) 407–412.

[44] S.V. Dorozhkin, M. Epple, Biological and medical significance of calcium phosphates, Angew. Chem. Int. Ed. 41 (2002) 3130–3146.

[45] L. Xiong, J. Zeng, A. Yao, Q. Tu, J. Li, L. Yan, Z. Tang, BMP2-loaded hollow hydroxyapatite microspheres exhibit enhanced osteoinduction and osteogenicity in large bone defects, Int. J. Nanomed. 10 (2015) 517–526.

[46] C. Wang, Y. Duan, B. Markovic, J. Barbara, C.R. Howlett, X.D. Zhang, H. Zreiqat, Proliferation and bone-related gene expression of osteoblasts grown on hydroxyapatite ceramics sintered at different temperature, Biomaterials 25 (2004) 2949–2956.

[47] M. Vallet-Regí, J.M. González-Calbet, Calcium phosphates as substitution of bone tissues, Prog. Solid State Chem. 32 (2004) 1–31.

[48] L. Wang, G.H. Nancollas, Calcium orthophosphates: crystallization and dissolution, Chem. Rev. 108 (2008) 4628–4669.

[49] F. Yao, J.P. Legeros, R.Z. Legeros, Simultaneous incorporation of carbonate and fluoride in synthetic apatites: Effect on crystallographic and physico-chemical properties, Acta Biomater. 5 (2009) 2169–2177.

[50] A. Bigi, E. Foresti, M. Gandolfi, M. Gazzano, N. Roveri, Isomorphous substitutions in β-tricalcium phosphate: the different effects of zinc and strontium, J. Inorg. Biochem. 66 (1997) 259–265.

[51] S. Kannan, I.A.F. Lemos, J.H.G. Rocha, J.M.F. Ferreira, Synthesis and characterization of magnesium substituted biphasic mixtures of controlled hydroxyapatite/β-tricalcium phosphate ratios, J. Solid State Chem. 178 (2005) 3190–3196.

[52] S.J. Saint-Jean, C.L. Camiré, P. Nevsten, S. Hansen, M.P. Ginebra, Study of the reactivity and in vitro bioactivity of Sr-substituted α-TCP cements, J. Mater. Sci. Mater. Med. 16 (2005) 993–1001.

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

[54] V. Mourino, J.P. Cattalini, A.R. Boccaccini, Metallic ions as therapeutic agents in tissue engineering scaffolds: an overview of their biological applications and strategies for new developments, J. R. Soc. Interface 9 (2011) 401–419.

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

[56] D. Xiao, F. Yang, Q. Zhao, S. Chen, F. Shi, X. Xiang, L. Deng, X. Sun, J. Weng, G. Feng, Fabrication of a Cu/Zn co-incorporated calcium phosphate scaffold-derived GDF-5 sustained release system with enhanced angiogenesis and osteogenesis properties, RSC Adv. 8 (2018) 29526–29534.

[57] E.S. Thian, Z. Ahmad, J. Huang, M.J. Edirisinghe, S.N. Jayasinghe, D.C. Ireland, R.A. Brooks, N. Rushton, W. Bonfield, S.M. Best, The role of surface wettability and surface charge of electrosprayed nanoapatites on the behaviour of osteoblasts, Acta Biomater. 6 (2010) 750–755.

[58] S.A. Redey, M. Nardin, D. Bernache-Assolant, C. Rey, P. Delannoy, L. Sedel, P.J. Marie, Behavior of human osteoblastic cells on stoichiometric hydroxyapatite and type A carbonate apatite: role of surface energy, J. Biomed. Mater. Res. 50 (2000) 353–364.

[59] K. Nishizawa, M. Toriyama, T. Suzuki, Y. Kawamoto, Y. Yokogawa, H. Nagae, Effects of the surface wettability and zeta potential of bioceramics on the adhesiveness of anchorage-dependent animal cells, J. Ferment. Bioeng. 75 (1993) 435–437.

[60] K. Ohta, H. Monma, S. Takahashi, Adsorption characteristics of proteins on calcium phosphates using liquid chromatography, J. Biomed. Mater. Res. 55 (2001) 409–414.

[61] X. Huang, Z. Wang, D. Li, Z. Huang, X. Dong, C. Li, J. Lan, Study of microRNAs targeted Dvl2 on the osteoblasts differentiation of rat BMSCs in hyperlipidemia environment, J. Cell. Physiol. 233 (2018) 6758–6766.

[62] M.A. Cleary, R. Narcisi, A. Albiero, F. Jenner, L.M. de Kroon, W.J. Koevoet, P.A. Brama, G.J. van Osch, Dynamic regulation of TWIST1 expression during chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells, Stem Cells Dev. 26 (2017) 751–761.

[63] X. Zhang, M.P. Bendeck, C.A. Simmons, J.P. Santerre, Deriving vascular smooth muscle cells from mesenchymal stromal cells: evolving differentiation strategies and current understanding of their mechanisms, Biomaterials 145 (2017) 9–22.

[64] D.S. Benoit, M.P. Schwartz, A.R. Durney, K.S. Anseth, Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells, Nat. Mater. 7 (2008) 816–823.

[65] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.

[66] L. Xia, K. Lin, X. Jiang, B. Fang, Y. Xu, J. Liu, D. Zeng, M. Zhang, X. Zhang, J. Chang, Effect of nano-structured bioceramic surface on osteogenic differentiation of adipose derived stem cells, Biomaterials 35 (2014) 8514–8527.

[67] T. Arahira, M. Todo, Effects of proliferation and differentiation of mesenchymal stem cells on compressive mechanical behavior of collagen/β-TCP composite scaffold, J. Mech. Behav. Biomed. Mater. 39 (2014) 218–230.

[68] A.B. Faia-Torres, S. Guimond-Lischer, M. Rottmar, M. Charnley, T. Goren, K. Maniura-Weber, N.D. Spencer, R.L. Reis, M. Textor, N.M. Neves, Differential regulation of osteogenic differentiation of stem cells on surface roughness gradients, Biomaterials 35 (2014) 9023–9032.

[69] Z. Tang, X. Li, Y. Tan, H. Fan, X.D. Zhang, The material and biological characteristics of osteoinductive calcium phosphate ceramics, Regen. Biomater. 5 (2017) 43–59.

[70] P. Habibovic, T.M. Sees, V.D.D. Ma, C.A. van Blitterswijk, G.K. De, Osteoinduction by biomaterials–physicochemical and structural influences, J. Biomed. Mater. Res. Part A 77A (2010) 747–762.

[71] J. Möller-Siegert, J. Parmentier, P. Laquerrière, A. Ouadi, O. Raisslé, E. Jallot, J.M. Nedelec, C. Vix-Guterl, K. Anselme, Physicochemical regulation of TGF and VEGF delivery from mesoporous calcium phosphate bone substitutes, Nanomedicine 12 (2017) 1835–1850.

[72] J. Zhang, M.T. Dalbay, X. Luo, E. Vrij, D. Barbieri, L. Moroni, J.D. de Bruijn, C.A. van Blitterswijk, J.P. Chapple, M.M. Knight, Topography of calcium phosphate ceramics regulates primary cilia length and TGF receptor recruitment associated with osteogenesis, Acta Biomater. 57 (2017) 487–497.

[73] B. Bhushan, Surface roughness analysis and measurement techniques, in: Modern Tribology Handbook, CRC Press, 2000, pp. 79–150.

[74] J. Li, G. Li, K. Zhang, Y. Liao, P. Yang, M.F. Maitz, N. Huang, Co-culture of vascular endothelial cells and smooth muscle cells by hyaluronic acid micro-pattern on titanium surface, Appl. Surf. Sci. 273 (2013) 24–31.

[75] J. Li, K. Zhang, P. Yang, Y. Liao, L. Wu, J. Chen, A. Zhao, G. Li, N. Huang, Research of smooth muscle cells response to fluid flow shear stress by hyaluronic acid micro-pattern on a titanium surface, Exp. Cell Res. 319 (2013) 2663–2672.

[76] T. Akasaka, H. Miyaji, N. Kaga, A. Yokoyama, S. Abe, Y.J. Yoshida, Adhesion of osteoblast-like cells (Saos-2) on micro-/submicro-patterned apatite scaffolds fabricated with apatite cement paste by micro-Molding, Nano Biomed. 8 (2016) 112–122.

[77] R. Song, R. Liang, L. Lin, Y. Zhang, Y. Yang, C. Lin, A facile construction of gradient micro-patterned OCP coatings on medical titanium for high throughput evaluation of biocompatibility, J. Mater. Chem. B 4 (2016) 4017–4024.

[78] A. Klymov, J. Song, X. Cai, J. te Riet, S. Leeuwenburgh, J.A. Jansen, X.F. Walboomers, Increased acellular and cellular surface mineralization induced by nanogrooves in combination with a calcium-phosphate coating, Acta Biomater. 31 (2016) 368–377.

[79] S.P. Yang, C.Y. Yang, T.M. Lee, T.S. Lui, Effects of calcium-phosphate topography on osteoblast mechanobiology determined using a cytodetacher, Mater. Sci. Eng. C 32 (2012) 254–262.

[80] L. Zhou, Y. Lai, W. Huang, S. Huang, Z. Xu, J. Chen, D. Wu, Biofunctionalization of microgroove titanium surfaces with an antimicrobial peptide to enhance their bactericidal activity and cytocompatibility, Colloids Surf. B 128 (2015) 552–560.

[81] M.G. Holthaus, J. Stolle, L. Treccani, K. Rezwan, Orientation of human osteoblasts on hydroxyapatite-based microchannels, Acta Biomater. 8 (2012) 394–403.

[82] X. Lu, Y. Leng, Comparison of the osteoblast and myoblast behavior on hydroxyapatite microgrooves, J. Biomed. Mater. Res. Part B Appl. Biomater. 90 (2009) 438–445.

[83] S. Pang, Y. He, P. He, X. Luo, Z. Guo, H. Li, Fabrication of two distinct hydroxyapatite coatings and their effects on MC3T3-E1 cell behavior, Colloids Surf. B 171 (2018) 40–48.

[84] D. Xiao, T. Guo, F. Yang, G. Feng, F. Shi, J. Li, D. Wang, K. Duan, J. Weng, In situ formation of nanostructured calcium phosphate coatings on porous hydroxyapatite scaffolds using a hydrothermal method and the effect on mesenchymal stem cell behavior, Ceram. Int. 43 (2017) 1588–1596.

[85] S. Choi, W.L. Murphy, The effect of mineral coating morphology on mesenchymal stem cell attachment and expansion, J. Mater. Chem. 22 (2012) 25288–25295.

[86] D.O. Costa, P.D.H. Prowse, T. Chrones, S.M. Sims, D.W. Hamilton, A.S. Rizkalla, S.J. Dixon, The differential regulation of osteoblast and osteoclast activity by surface topography of hydroxyapatite coatings, Biomaterials 34 (2013) 7215–7226.

[87] L. Xia, K. Lin, X. Jiang, Y. Xu, M. Zhang, J. Chang, Z. Zhang, Enhanced osteogenesis through nano-structured surface design of macroporous hydroxyapatite bioceramic scaffolds via activation of ERK and p38 MAPK signaling pathways, J. Mater. Chem. B 1 (2013) 5403–5416.

[88] C. Zhao, X. Wang, L. Gao, L. Jing, Q. Zhou, J. Chang, The role of the micro-pattern and nano-topography of hydroxyapatite bioceramics on stimulating osteogenic differentiation of mesenchymal stem cells, Acta Biomater. 73 (2018) 509–521.

[89] D.D. Deligianni, N.D. Katsala, P.G. Koutsoukos, Y.F. Missirlis, Effect of surface roughness of hydroxyapatite on human bone marrow cell adhesion, proliferation, differentiation and detachment strength, Biomaterials 22 (2000) 87–96.

[90] X. Zan, P. Sitasuwan, S. Feng, Q. Wang, Effect of roughness on in situ biomineralized CaP-collagen coating on the osteogenesis of mesenchymal stem cells, Langmuir 32 (2016) 1808–1817.

[91] M.L. Cairns, B.J. Meenan, G.A. Burke, A.R. Boyd, Influence of surface topography on osteoblast response to fibronectin coated calcium phosphate thin films, Colloids Surf. B Biointerfaces 78 (2010) 283–290.

[92] K. Anselme, Osteoblast adhesion on biomaterials, Biomaterials 21 (2000) 667–681.

[93] H. Zeng, K.K. Chittur, W.R. Lacefield, Analysis of bovine serum albumin adsorption on calcium phosphate and titanium surfaces, Biomaterials 20 (1999) 377–384.

[94] D.I. Hay, E.C. Moreno, Differential adsorption and chemical affinities of proteins for apatitic surfaces, J. Dent. Res. 58 (1979) 930–942.

[95] D.T.H. Wassell, R.C. Hall, G. Embery, Adsorption of bovine serum albumin onto hydroxyapatite, Biomaterials 16 (1995) 697–702.

[96] Q. Luo, J.D. Andrade, Cooperative adsorption of proteins onto hydroxyapatite, J. Colloid Interface Sci. 200 (1998) 104–113.

[97] Y. Chen, X. Zhang, Y. Gong, N. Zhao, T. Zeng, X. Song, Conformational changes of fibrinogen adsorption onto hydroxyapatite and titanium oxide nanoparticles, J. Colloid Interface Sci. 214 (1999) 38–45.

[98] E.A. Dos Santos, M. Farina, G.A. Soares, K. Anselme, Surface energy of hydroxyapatite and β-tricalcium phosphate ceramics driving serum protein adsorption and osteoblast adhesion, J. Mater. Sci. Mater. Med. 19 (2008) 2307–2316.

[99] M. Han, A. Sethuraman, R.S. Kane, G. Belfort, Nanometer-scale roughness having little effect on the amount or structure of adsorbed protein, Langmuir 19 (2003) 9868–9872.

[100] F.L. Yap, Y. Zhang, Protein and cell micropatterning and its integration with micro/nanoparticles assembly, Biosens. Bioelectron. 22 (2007) 775–788.

[101] P. Roach, D. Farrar, C.C. Perry, Surface tailoring for controlled protein adsorption: effect of topography at the nanometer scale and chemistry, J. Am. Chem. Soc. 128 (2006) 3939–3945.

[102] C. Combes, C. Rey, Adsorption of proteins and calcium phosphate materials bioactivity, Biomaterials 23 (2002) 2817–2823.

[103] M. Nawata, S. Wakitani, H. Nakaya, A. Tanigami, T. Seki, Y. Nakamura, N. Saito, K. Sano, E. Hidaka, K. Takaoka, Use of bone morphogenetic protein 2 and diffusion chambers to engineer cartilage tissue for the repair of defects in articular cartilage, Arthritis Rheum. 52 (2005) 155–163.

[104] X.D. Zhu, H.S. Fan, Y.M. Xiao, D.X. Li, H.J. Zhang, T. Luxbacher, X.D. Zhang, Effect of surface structure on protein adsorption to biphasic calcium-phosphate ceramics in vitro and in vivo, Acta Biomater. 5 (2009) 1311–1318.

[105] S.M. Pockwinse, J.L. Stein, J.B. Lian, G.S. Stein, Developmental stage-specific cellular responses to vitamin D and glucocorticoids during differentiation of the osteoblast phenotype: Interrelationship of morphology and gene expression by in situ hybridization, Exp. Cell Res. 216 (1995) 244–260.

[106] V.I. Sikavitsas, J.S. Temenoff, A.G. Mikos, Biomaterials and bone mechanotransduction, Biomaterials 22 (2001) 2581–2593.

[107] R. Carvalho, J. Schaffer, L. Gerstenfeld, Osteoblasts induce osteopontin expression in response to attachment on fibronectin: demonstration of a common role for integrin receptors in the signal transduction processes of cell attachment and mechanical stimulation, J. Cell. Biochem. 70 (1998) 376–390.

[108] J.L.R. Fernández, A. Ben-Ze'ev, Regulation of fibronectin, integrin and cytoskeleton expression in differentiating adipocytes: Inhibition by extracellular matrix and polylysine, Differentiation 42 (1989) 65–74.

[109] J.T. Eggenschwiler, K.V. Anderson, Cilia and developmental signaling, Annu. Rev. Cell Dev. Biol. 23 (2007) 345–373.

[110] R.J. McMurray, A.K.T. Wann, C.L. Thompson, J.T. Connelly, M.M. Knight, Surface topography regulates wnt signaling through control of primary cilia structure in mesenchymal stem cells, Sci. Rep. 3 (2013) 3545.

[111] J.C. Bodle, C.D. Rubenstein, M.E. Phillips, S.H. Bernacki, J. Qi, A.J. Banes, E.G. Loboa, Primary cilia: the chemical antenna regulating human adipose-derived stem cell osteogenesis, PLoS One 8 (2013) e62554.

[112] J.C. Wu, Y.C. Chen, C.T. Kuo, H.W. Yu, Y.Q. Chen, A. Chiou, J.C. Kuo, Focal adhesion kinase-dependent focal adhesion recruitment of SH2 domains directs SRC into focal adhesions to regulate cell adhesion and migration, Sci. Rep. 5 (2015) 18476.

[113] S.L. Vega, V. Arvind, P. Mishra, J. Kohn, N.S. Murthy, P.V. Moghe, Substrate micropatterns produced by polymer demixing regulate focal adhesions, actin anisotropy, and lineage differentiation of stem cells, Acta Biomater. 76 (2018) 21–28.

[114] M.N. Andalib, J.S. Lee, L. Ha, Y. Dzenis, J.Y. Lim, Focal adhesion kinase regulation in stem cell alignment and spreading on nanofibers, Biochem. Biophys. Res. Commun. 473 (2016) 920–925.

[115] D.G. Bello, A. Fouillen, A. Badia, A. Nanci, A nanoporous titanium surface promotes the maturation of focal adhesions and formation of filopodia with distinctive nanoscale protrusions by osteogenic cells, Acta Biomater. 60 (2017) 339–349.

[116] Y. Sun, W.Z. Liu, T. Liu, X. Feng, N. Yang, H.F. Zhou, Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis, J. Recept. Signal Transduct. 35 (2015) 600–604.

[117] S. Haston, S. Pozzi, G. Carreno, S. Manshaei, L. Panousopoulos, J.M. Gonzalez-Meljem, J.R. Apps, A. Virasami, S. Thavaraj, A. Gutteridge, MAPK pathway control of stem cell proliferation and differentiation in the embryonic pituitary provides insights into the pathogenesis of papillary craniopharyngioma, Development 144 (2017) 2141–2152.

[118] J. Yang, L.E. McNamara, N. Gadegaard, E.V. Alakpa, K.V. Burgess, R.D. Meek, M.J. Dalby, Nanotopographical induction of osteogenesis through adhesion, bone morphogenic protein cosignaling, and regulation of microRNAs, ACS Nano 8 (2014) 9941–9953.

[119] L. Mao, J. Liu, J. Zhao, J. Chang, L. Xia, L. Jiang, X. Wang, K. Lin, B. Fang, Effect of micro-nano-hybrid structured hydroxyapatite bioceramics on osteogenic and cementogenic differentiation of human periodontal ligament stem cell via Wnt signaling pathway, Int. J. Nanomed. 10 (2015) 7031–7044.

[120] W. Yang, W. Han, W. He, J. Li, J. Wang, H. Feng, Y. Qian, Surface topography of hydroxyapatite promotes osteogenic differentiation of human bone marrow mesenchymal stem cells, Mater. Sci. Eng. C 60 (2016) 45–53.

[121] H. Pan, Y. Xie, Z. Zhang, K. Li, D. Hu, X. Zheng, Q. Fan, T. Tang, YAP-mediated mechanotransduction regulates osteogenic and adipogenic differentiation of BMSCs on hierarchical structure, Colloids Surf. B 152 (2017) 344–353.

[122] W. Liu, Y. Wei, X. Zhang, M. Xu, X. Yang, X. Deng, Lower extent but similar rhythm of osteogenic behavior in hBMSCs cultured on nanofibrous scaffolds versus induced with osteogenic supplement, ACS Nano 7 (2013) 6928–6938.

[123] M.R. Appleford, S. Oh, J.A. Cole, D.L. Carnes, M. Lee, J.D. Bumgardner, W.O. Haggard, J.L. Ong, Effects of trabecular calcium phosphate scaffolds on stress signaling in osteoblast precursor cells, Biomaterials 28 (2007) 2747–2753.

[124] T. Ono, H. Takayanagi, Osteoimmunology in bone fracture healing, Curr. Osteoporos. Rep. 15 (2017) 367–375.

[125] T. Ono, K. Okamoto, T. Nakashima, T. Nitta, S. Hori, Y. Iwakura, H. Takayanagi, IL-17-producing $\gamma\delta$ T cells enhance bone regeneration, Nat. Commun. 7 (2016) 10928.

[126] N. Davison, X. Luo, T. Schoenmaker, V. Everts, H. Yuan, F. Barrere-de Groot, J.D. de Bruijn, Submicron-scale surface architecture of tricalcium phosphate directs osteogenesis in vitro and in vivo, Eur. Cells Mater. 27 (2014) 281–297.

[127] X. Chen, J. Wang, X. Zhu, X. Yang, Y. Fan, X.D. Zhang, The positive role of macrophage secretion stimulated by BCP ceramic in the ceramic-induced osteogenic differentiation of pre-osteoblasts via Smad-related signaling pathways, RSC Adv. 6 (2016) 102134–102141.

[128] J. Wang, D. Liu, B. Guo, X. Yang, X. Chen, X. Zhu, Y. Fan, X.D. Zhang, Role of biphasic calcium phosphate ceramic-mediated secretion of signaling molecules by macrophages in migration and osteoblastic differentiation of MSCs, Acta Biomater. 51 (2017) 447–460.

[129] Z. Chen, T. Klein, R.Z. Murray, R. Crawford, J. Chang, C. Wu, Y. Xiao, Osteoimmunomodulation for the development of advanced bone biomaterials, Mater. Today 19 (2016) 304–321.

[130] Z. Chen, S. Ni, S. Han, R. Crawford, S. Lu, F. Wei, J. Chang, C. Wu, Y. Xiao, Nanoporous microstructures mediate osteogenesis by modulating the osteo-immune response of macrophages, Nanoscale 9 (2017) 706–718.

[131] N.E. Paul, C. Skazik, M. Harwardt, M. Bartneck, B. Denecke, D. Klee, J. Salber, G. Zwadlo-Klarwasser, Topographical control of human macrophages by a regularly microstructured polyvinylidene fluoride surface, Biomaterials 29 (2008) 4056–4064.

[132] X. Wu, Y. Dai, Y. Yang, C. Huang, X. Meng, B. Wu, J. Li, Emerging role of micro RNA s in regulating macrophage activation and polarization in immune response and inflammation, Immunology 148 (2016) 237–248.

[133] T.U. Luu, S.C. Gott, B.W. Woo, M.P. Rao, W.F. Liu, Micro-and nanopatterned topographical cues for regulating macrophage cell shape and phenotype, ACS Appl. Mater. Interfaces 7 (2015) 28665–28672.

[134] Q. Huang, J.W. Hwang, J.H. Oh, C.H. Park, S.H. Chung, Y.S. Lee, J.H. Baek, H.M. Ryoo, K.M. Woo, Effects of the fibrous topography-mediated macrophage phenotype transition on the recruitment of mesenchymal stem cells: an in vivo study, Biomaterials 149 (2017) 77–87.

[135] N.L. Davison, A.L. Gamblin, P. Layrolle, H. Yuan, J.D. de Bruijn, F. Barrère-de Groot, Liposomal clodronate inhibition of osteoclastogenesis and osteoinduction by submicrostructured beta-tricalcium phosphate, Biomaterials 35 (2014) 5088–5097.

[136] N.L. Davison, B. ten Harkel, T. Schoenmaker, X. Luo, H. Yuan, V. Everts, F. Barrère-de Groot, J.D. de Bruijn, Osteoclast resorption of beta-tricalcium phosphate controlled by surface architecture, Biomaterials 35 (2014) 7441–7451.

[137] R. Jimbo, Y. Xue, M. Hayashi, H.O. Schwartz-Filho, M. Andersson, K. Mustafa, A. Wennerberg, Genetic responses to nanostructured calcium-phosphate-coated implants, J. Dent. Res. 90 (2011) 1422–1427.