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The modulation of stem cell behaviors by functionalized nanoceramic coatings on Ti-based implants

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ABSTRACT

Nanoceramic coating on the surface of Ti-based metallic implants is a clinical potential option in orthopedic surgery. Stem cells have been found to have osteogenic capabilities. It is necessary to study the influences of functionalized nanoceramic coatings on the differentiation and proliferation of stem cells *in vitro* or *in vivo*. In this paper, we summarized the recent advance on the modulation of stem cells behaviors through controlling the properties of nanoceramic coatings, including surface chemistry, surface roughness and microporosity. In addition, mechanotransduction pathways have also been discussed to reveal the interaction mechanisms between the stem cells and ceramic coatings on Ti-based metals. In the final part, the osteoinduction and osteoconduction of ceramic coating have been also presented when it was used as carrier of BMPs in new bone formation.

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1. Introduction

The proportion of orthopedic in surgery is growing with the development of orthomorphia and the increasing rate of fracture or joint degeneration which is the result of the aging population. New long-term implants are more strongly required nowadays. It is predicted that the amount of total hip implants will grow by 174%–572,000 procedures while the demand of total knee arthroplasties estimates to rise by 673% to 3.48 million by 2030 [1]. It has been reported that more than 500,000 bone graft surgeries happen in America and about 2.2 million throughout the world (these surgeries cost approximately 2.5 billion dollars each year) are performed to address bone fractures and other orthopedic-related injuries resulting from a variety of surgical [2,3], traumatic and degenerative causes [4]. Both the autoplastic transplantation and allograft from human living donors or cadavers are used in orthopedics transplantation. And xenograft bone from a non-human also can be an available graft in orthopedic operation. Currently, the best choice in bone graft treatment is autologous bone, harvested primarily from the patients' iliac crest or other parts like the proximal tibia, intramedullary canal, distal femur, and ribs [2,4–6]. Though the autograft bone is at an advantage of its immunocompatibility and fantabulous osteoconductive properties, it cannot be widely used for the sake of donor shortages. Metallic biomaterials are often used in orthopedic devices for the fixation and immobilization of bone fractures to meet the requirements of maintaining mechanical integrity and biocompatibility during the healing process. Due to their superior biocompatibility, relatively low modulus of elasticity, high mechanical strength and some other suitable mechanical properties [7], titanium and its alloys have been used extensively in biomedical fields for orthopedic applications under load-bearing conditions [8]. However, titanium and its alloys have been proven to be bioinert and they cannot have a direct chemical bonding to living bone tissues [9].

Researchers have done plenty of surface modifications to ameliorate the biological, chemical and mechanical properties of Ti and its alloys in the last decades. A series of surface engineering techniques, including plasma spraying, microarc oxidation, sol-gel, electrochemical deposition and laser cladding and etc. [10–14], were used to fabricate bioactive nanoceramic coatings on the surface of these metals. Nanoceramics have excellent performances, including high mechanical strength, superior tribological property, bioactivity and resorbability [2]. Nano calcium phosphate ceramics such as hydroxyapatite (HA), α-tricalcium phosphate (α-TCP), β-tricalcium phosphate (β-TCP) and tetracalcium phosphate [9,15,16] show good bone-bonding to natural bones. Macropores on or inside the ceramic can intensify the ingrowth of tissue and accelerate the degrading process of ceramic as well [15].

Table 1 enumerates the advantages and disadvantages of autogenous bone, allograft bone, heterogenous bone, Ti alloys and Ti alloys with nanoceramic coatings [7,9,13,14]. Among the ceramics mentioned above, HA coatings have the most similar ingredients to human bones. Once the biomaterials are transplanted into body to replace the defect sites, the implants inevitably attach with surrounding tissue and may react with them. Table 2 gives types of bioceramic-tissue attachments [17].

Tissue engineering applied concepts and methods which are from both engineering and life sciences to maintain existing tissue or to promote regeneration of new tissue [18]. Based on the understanding of tissue formation and regeneration, tissue engineering proposes to induce the formation of new functional tissues rather than to implant new spare parts compared to classical biomaterials methods [19]. The alleged triplets in tissue engineering comprehend three essential components: scaffolds, cells and signaling biomolecules (or growth factors). Among them, the cells, which are widely cognized as the parts of an engineered tissue or absorbed onto/inside the scaffolds, compose the “prototype” of the living tissue to generate and to synthesize matrices for repopulation. After the implants transplanted into human body, stem cells play vital roles in restoring new bones. According to the stage of development, stem cells can be divided into two types: embryonic stem cells (ESCs) and somatic stem cells. All kinds of stem cells such as ESCs, mesenchymal stem cells (MSCs, derive from bone marrow or umbilical cord blood), adipose tissue-derived stem cells (ADSCs), muscle-derived stem cells (MDSCs) and dental pulp stem cells (DPSCs) [20] are used in bone tissue engineering for osteogenic differentiation owing to their potential of differentiate into different lineages after suitable stimuli.

Therefore, in the following sections, we will firstly generalize these proper stimuli which come from the surface characters of nanoceramic coatings on Ti and its alloys, including the surface chemistry, surface roughness and microporosity, and their corresponding effects on the differentiation behaviors of stem cells will also be discussed as well.

2. Effects of surface chemistry

2.1. Protein adsorption

Protein adsorption in the differentiation and proliferation of stem cells is a complex process. Surface charge, ionic environment and solubility of substrates can play important roles in protein adsorption. Nanophase hydroxyapatite (HA) is a nano-scaled needle-like crystal with circa 5–20 nm in width and 60 nm in length,

Table 1
Comparisons of different implant materials [7,9,13,14].

Type of implants	Advantages	Disadvantages
Autograft bone	Immunecompatibility and fantabulous osteoconduction	Donor shortages
Allograft bone	Osteoconduction	Transfer of infection and antigenicity
Heterogenous bone	Sufficient source	Immunorejection
Ti alloys	Biocompatibility, relatively low modulus of elasticity, high mechanical strength	Indirect chemical bonding to living bone tissues
Ti alloys with nanoceramic coatings [9] [13] [14]	Good bone-bonding to natural bones	Adhesion strength needs to be improved

Table 2
Types of ceramic-tissue attachments [17].

Bioceramic type	Type of attachment	Characters of ceramic
1	Attach by bone growth into surface irregularities (morphological fixation)	Dense, nonporous, nearly inert
2	Mechanically attaches the bone to the material (biological fixation)	Porous, inert
3	Chemical bonding with the bone (bioactive fixation)	Dense, nonporous, bioactive
4	Slowly replaced by bone	Dense, resorbable

and its surface grain size, pore size, wettability and other characteristics could heighten osteoblast adhesion and long-term functionality by adjusting the mutual influences of proteins [21]. The nanosized surface can mimic the natural bone surface for osteogenic proliferation and differentiation. A nano-TiO_2/HA composite bioceramic coating, which has nano scale porous surface morphology, was used *in vitro* osteoblast cultures [22]. Compared with the conventional HA (m-HAP), nano HA showed more cells in osteoblast culture experiment after 5 days (np80 is 215 ± 21 mm^{-2} while m-HAP is 135 ± 13 mm^{-2}) [23]. It has been reported that hydroxyl (-OH) surface on HA can exhibit higher binding affinity with $\alpha_5\beta_1$ integrin [24]. The binding of integrin effect changes in fibronectin structure. On the other hand, the neutral hydrophilic moiety OH sided a highest supplementary level for proteins (such as talin, α-actinin, paxillin, and tyrosine-phosphorylated protein), which are related to the cell adhesion.

2.2. Cell proliferation and differentiation

It has been demonstrated that there is an effect of Mg^{2+} and Zn^{2+} -containing silicate-based bioceramic on the osteostimulation of periodontal ligament cells (PDLCs) and bone marrow-derived mesenchymal stem cells (BMSCs) [25]. Diopside (DIOP: $CaMgSi_2O_6$) was used as the carrier of Mg^{2+} and hardystonite (HT: $Ca_2ZnSi_2O_7$) was used as the carrier of Zn^{2+}. The Zn^{2+} positively affected the proliferation of both PDLCs and BMSCs only at a low concentration. Middle concentration of Mg^{2+} induced higher expression of OCN in BMSCs. Hu et al. [26] used rat bone marrow stem cells (bMSCs) to investigate the innovative Zn-incorporated TiO_2 coatings. The proliferation of bMSCs has been enhanced by the incorporation of Zn.

In the research of Thian et al. [27], two-dimensional ceramic microstructures were fabricated to investigate the effects of surface chemistry on osteoblast outgrowth. The number of osteoblasts spread on nanoscaled silicon-substituted hydroxyapatite was significantly increased compared to nanoscaled carbonate-substituted hydroxyapatite. Gough et al. reported that ion environment play roles in apoptosis in cell proliferation and a 9% decrease in levels of apoptosis when cells cultured in neutralized bioactive glass dissolution [28].

In summary, the surface chemistry could influence the behaviors of stem cells by adjusting the scaffold compositions.

3. Effects of surface roughness

The improvement of requisite interface is strongly influenced by surface chemistry as well as nanoscale and microscale topographies. Bone resorption by osteoclasts is accompanied by deposition of calcium-containing mineral by osteoblasts *in vivo*. Compared with the conventional ceramics, the synthesis of tartrate-resistant acid phosphatase (TRAP) and formation of resorption pits was significantly greater in osteoclast-like cells cultured on nanophase ceramics, such as nanophase alumina and nanophase HA [29]. Roughness is a quantitative measure of surface texture and is usually valued by R_a, a root mean square value. R_a describes the distance between the troughs and crests through a straight line on the surface of substance [30]. Roughness is related to the grain size of nanophase ceramic crystallites and the size of nanophase ceramic particles, which play an important role for the modulation of cell behaviors. For instance, the adsorption of ECM proteins (such as vitronectin, fibrinogen and fibronectin) acts as pivotal roles in cell-adhesion onto synthetic surfaces as well as promotes the tissue regeneration [31]. The structure of these proteins changes when contacting with the matrix and subsequently affects the adhesion, mobility of cells.

3.1. Protein adsorption

As we know, surface roughness relies on the grain sizes of nanoceramic coating and particle size. In this section, some comparisons between conventional and nanoscale materials are presented. A recent research systematically investigated the effect of the hydroxyapatite bioceramics with distinct nanostructured topographies on protein adsorption, and in this work, bioceramic surface with nanomicro topographic structures exhibited a stronger band integrated intensities as well as the higher protein adsorption compared to the flat and dense surface (shown in Fig. 1) [32]. Webster et al. [21] have reported that in comparison with conventional ceramic HA (grain size of 179 nm and 10 nm R_a value), a stronger adsorption of proteins such as albumin, collagen and vitronectin has been shown on nanophase ceramic HA with a grain size of 67 nm and R_a value of 17 nm, respectively. In addition, another research raised by He et al. [33] have shown a positive trend that nano-scaled HA with grain size of 80–120 nm and micro-scaled HA with grain size of 200–400 nm increased the albumin adsorption, relating to conventional HA with grain size of 1.0–2.0 μm. Meanwhile, they have observed that more protein adsorption on the surface of nanoscaled HA than those of the other two HA ceramic materials, significantly.

In summary, these studies reveal that surface coatings with smaller feature sizes, including surface roughness, grain size and particle size, may adsorb more proteins than that with larger

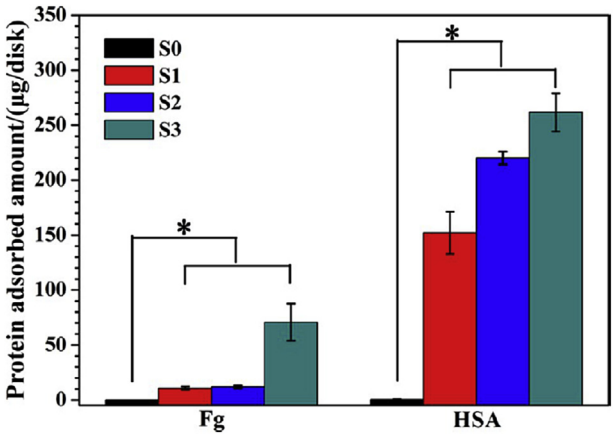

Fig. 1. Two kinds of proteins, Fg and HSA, adsorption on control sample (S0, R value is 0.372) and the fabricated HAp bioceramics with differently topographic surfaces: nanosheets (S1, R value is 0.376), nanorods (S2, R value is 0.292), and micro-nanohybrid (S3, R value is 2.004). *$p < 0.05$ [32].

feature sizes.

3.2. Cell adhesion

The surface roughness had significant effect on the adsorption of protein, but effects of the nano- and submicron-scale roughness on cell adhesion seem not appear to be consistent. For example, Dulgar-Tulloch et al. [34] reported that nanophase ceramic surface with grain size of 50–100 nm reduced the adhesion of hMSCs compared to the grain size whose range was above 200 nm. Furthermore, their results also showed that hMSCs adhesion did not rely on the grain size when it was larger than 200 nm. Deligianni et al. found that HA disc with higher R_a (4.6 μm) revealed better BMSCs attachment than that of lower R_a value of 0.73 μm, as may be seen in Fig. 2, respectively [35]. Meanwhile, the percent of BMSCs attachment on HA disc with R_a value of 4.6 μm had a significant enhancement with the incubation time [35]. However, there was an opposite observation in an early investigation of BMSCs adhesion on fluoro-HA (which was as a coating on titanium alloy) [36]. Only slight distinction was detected in BMSCs adhesion on two different roughness fluoro-HA (the higher value of R_a was 21.2 μm and the lower value of R_a was 5.6 μm) [36]. A similar result could be found in the report of Montanaro et al. [37] who used the MG63 osteoblast-like cells in his experiments.

In conclusion, the effect of surface roughness of ceramic coating on stem cells adhesion has not been confirmed, which will need further investigation systematically.

4. Surface topography effects on cell behaviors

The ion environment and chemical compositions of metallic implants as well as the surface topography of the substrate effect cell by regulating their shape, adhesion to substrates, migration, proliferation and differentiation. Hence, patterning (well-defined topography) of the substrate becomes an effective tool to modulate the correlative cell responses [38,39]. In this section, we will introduce the effects of coating patterns, including grooves, pillars and pores on the cell behaviors.

In general, porous coating promotes bone cell adhesion, which could affect proliferation and differentiation of stem cells positively, as well as protein adsorption on the surface of implant materials that gives rise to a better bonding between implants and surrounding tissue. Micropores can enhance the circulation of interstitial fluid between macropores and micropores.

4.1. Cell shape

Topography features affect cell adhesion and alignment on the substrate [39–41]. Contact guidance of cells might be the famous phenomenon, which means cell alignment on an anisotropic surface. Generally, contact guidance leads cells to elongate along ridge axes [42]. In a study of Teixeira et al. human corneal epithelial cells elongated along grooves with 70 nm wide ridges and 600 nm depth on silicon oxide substrate, while most of cells presented a round shape on smooth substrates, and they also found that a constant percentage of aligned cells on substrate with lateral dimensions ranging from 0.4 to 4 μ m, while increased with groove depth [43]. Recently, Richert et al. employed a facile chemical oxidation method to obtain a network of nanopits, and osteogenic cells widely spread on the nanotextured surface that occupied around 80% more than on the controls [44]. Wittenbrink et al. found that osteoblast-like MG-63 cells and their pseudopodia were better aligned parallel to ripple pattern with a periodicity of 179 nm than on nanoripples with 24 nm periodicity and flat surfaces on alumina substrate, as shown in Fig. 3, suggesting that cells can sense well the sub-nm surface topography [45].

Huo et al. [46] showed titanium nanotubes with diameter of 30 nm and 80 nm dramatically promote cell extension forming the typical osteoblastic shape, while cells spread relatively poorly with a spindle shape on the flat Ti. In their research, cells extend on nanotube arrays with isotropy. The Ding group [47] reported that the contact guidance responses are physical rather than biochemical induction factors. So both physical and biological induction factors control the contact guidance of cells. As shown in Fig. 4, cell shape is an inherent cue to regulate stem cell differentiation in ROCK pathway [47].

4.2. Cell adhesion and proliferation

Cell adhesion was also affected by topography features and depends on the topography types. For instance, micropores impact the adhesion of stem cells in osteogenic differentiation. The effect of ceramic coating microporosity on cell adhesion always relates to that of macroporosity in many published reports. Bignon et al. [48] used porogen to obtain macroporosities and microporosities in bone ceramics to observe the cellular responses (osteoblasts were used in this experiment) to bone substitutes. The size of macroporosity and microporosities ranges from 2 to 80 μm and 0.3–2 μm, respectively. They found that the microporosity promote the cytoplasmic extension to enhance the cell spreading [48]. In the report of Rouahi et al., SaOs-2 (a kind of osteoblast) were cultured on both microporous hydroxyapatite (the diameter of the pores valued about 0.4 μm) and nonmicroporous hydroxyapatite ceramic for a comparison, and their result showed that the osteoblasts were excellently “adsorbed” by the microporous hydroxyapatite compared to the nonmicroporous hydroxyapatite [49]. This could

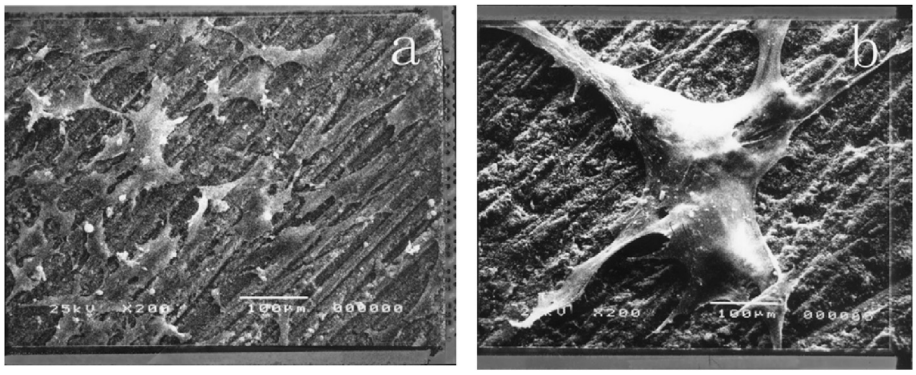

Fig. 2. Scanning electron microscope (SEM) micrograph showing the bone marrow cell morphology on a typical HA disc, polished with SiC metallographic paper a) 1200-grit, b) 600-grit, after 4 h of incubation [35].

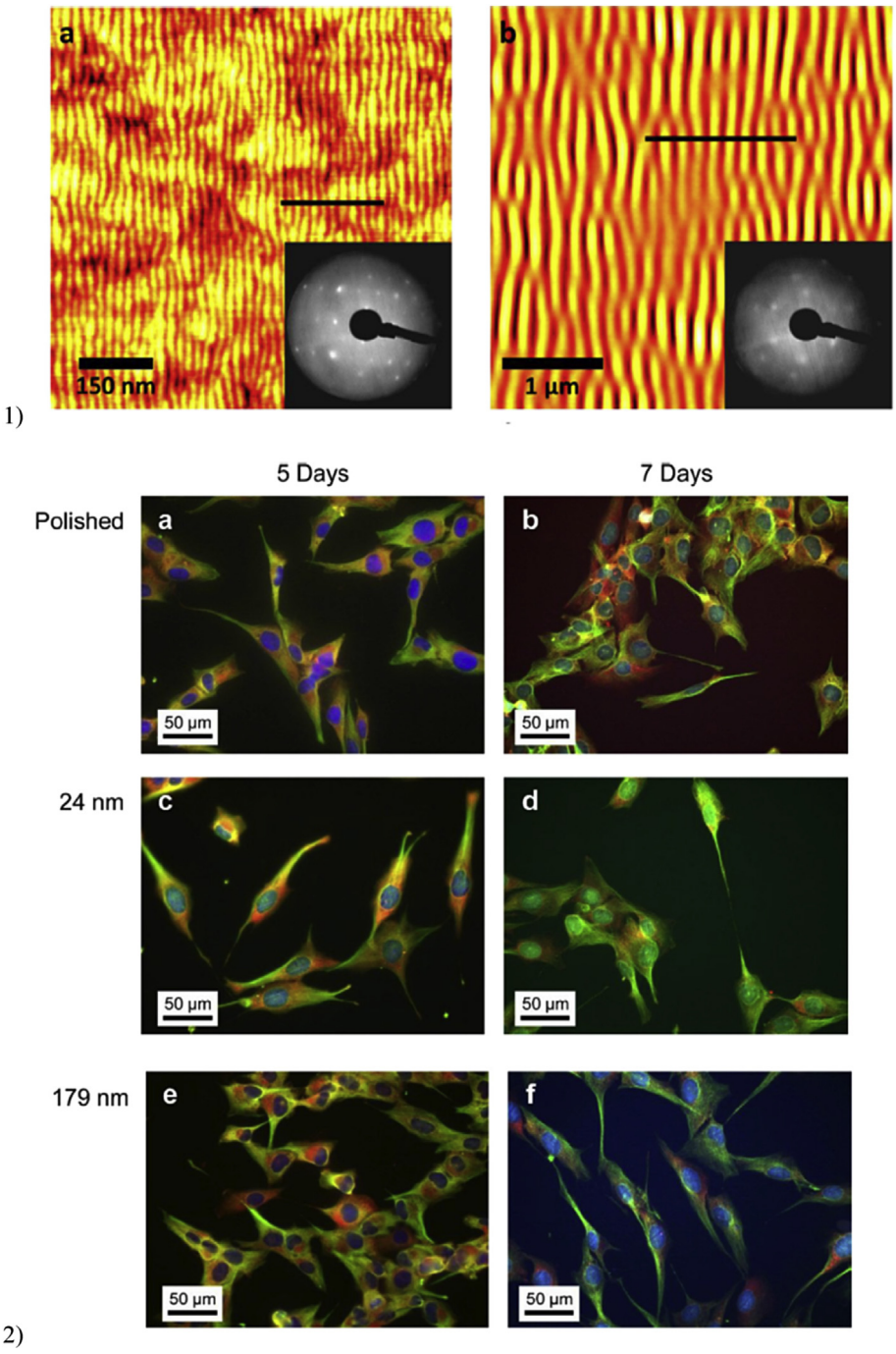

Fig. 3. 1) Atomic force microscope (AFM) images of nanopatterned Al_2O_3 surfaces with 24 nm (a) and 179 nm periodicity (b), 2) MG-63 cells were seeded on polished sapphire (a,b), 24 nm ripple patterns (c,d), and 179 nm ripple patterns (e,f), 3) SEM images of pseudopodia of MG-63 cells seeded on (a) polished alumina and (b,c) ripple patterns with (b) 24 nm and (c) 179 nm periodicity, respectively [45].

be getting less stark with the increasing of time in culture Fig. 5 exhibited images of the scanning electron microscope of material surfaces as well as Saos-2 osteoblastic cells cultured on for 30 min or 4 h of both microporous hydroxyapatite and nonmicroporous hydroxyapatite [49]. The same result also can be found in the work of Rouahi et al. [50]. They even gave several data of the number of attached cells on the mHA surface; those were 12-fold higher after 30 min, 6.7-fold higher after 1 h, 4.3-fold higher after 4 h and 2-fold higher after 24 h, compared with pHA. Overall, microporous facilitate the cytoplasmic extension for cell adhesion [50].

Topography features play roles in cell proliferation as well. Nanostructures accelerate the cell proliferation was reported by Wittenbrink et al. [45], and in their work, the 179 nm periodicity pattern showed a significantly higher induced enhancement of cell proliferation compared to the polished samples and 24 nm nanopatterned alumina surfaces. On the contrast, though the growth rates of fibroblasts on silicon surfaces of pits with diameter 7 μm were significant higher than plain surfaces and diameter of ~20 μm, the proliferation of the cells on the patterned surfaces of pits with diameter of 15 μm was not enhanced compared with 25 μm, as reported by Berry et al. [51]. These differences might be correlated with dimensions of surface features and cell types.

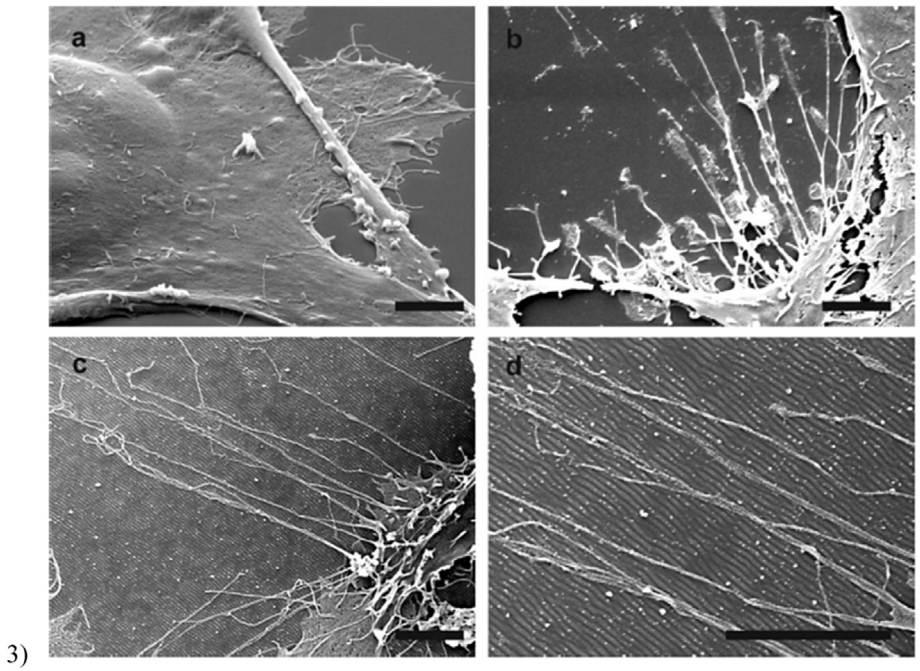

Fig. 3. (*continued*).

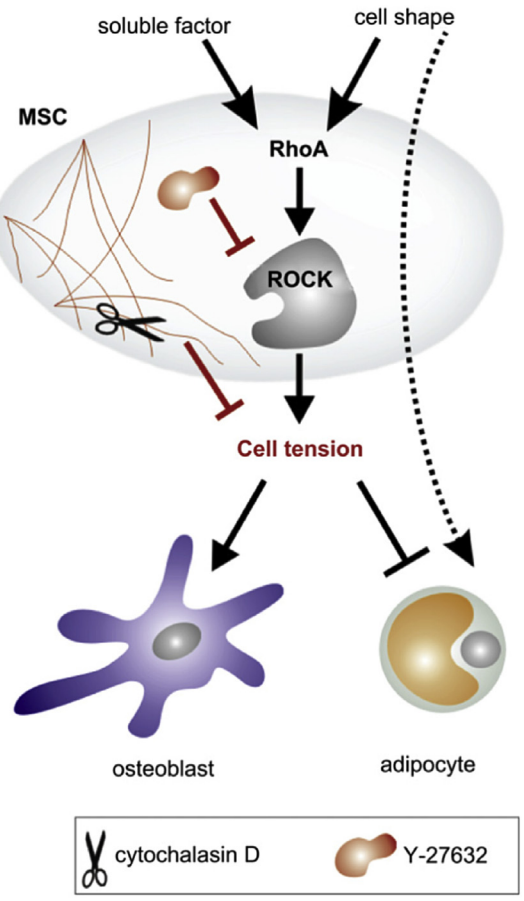

Fig. 4. The speculative pathways for cell-shape-directed osteogenic and adipogenic differentiations of MSCs examined in growth medium [47].

4.3. Cell migration

Migration is a basic cellular behavior. The effect of topography on migration can be typically observed in cells culture. Cell migration was related to several types of taxis. Chemotaxis is induced by chemical gradient, haptotaxis is mediated by immobilized ligand gradients, mechanotaxis is caused by mechanical force, durotaxis is mediated by matrix rigidity, and tensotaxis is induced by substrate strain [52]. Cell migration is a key cue in tissue induction, in which external cell can be seed into porous scaffolds, and the migration of internal cells thus is necessary for a successful tissue regeneration [53].

Shah et al. [54] co-cultured osteoblasts with endothelial cells onto the HA/PLA composite scaffolds in order to investigate the migration of the both cells. After 3 and 5 days culture, both cells are statistically significant increase in migration from HA ring into PLA plug. The migration of cells might be related to several factors, including the gradients of the topographic patterns and the taxis types.

4.4. Cell differentiation

The existence of microporous structure extents the specific surface area. And the three-dimensional porous structure of nanophase ceramic is more favorable for protein adsorption. Zhu et al. have investigated the interaction between protein adsorption and the porous structure of two kinds of BCP (porous BCP and dense BCP). In this study, porous BCP with ~100–500 μm diameter micropores displayed a higher adsorption of TGF-β1 than the dense BCP with low microporous distribution [55]. Microporosity has a connection with microenvironment. It may affect the binding between materials and protein, which could induce the attachment and osteogenic differentiation of cells. Another research have showed a similar result that Habibovic et al. [56] also investigated the osteoinduction of BCP and HA with different microporosity (which obtained by varying the sintering temperatures) *in vivo*. It has been found that microporosity (pore diameter < 10 μm) boosts

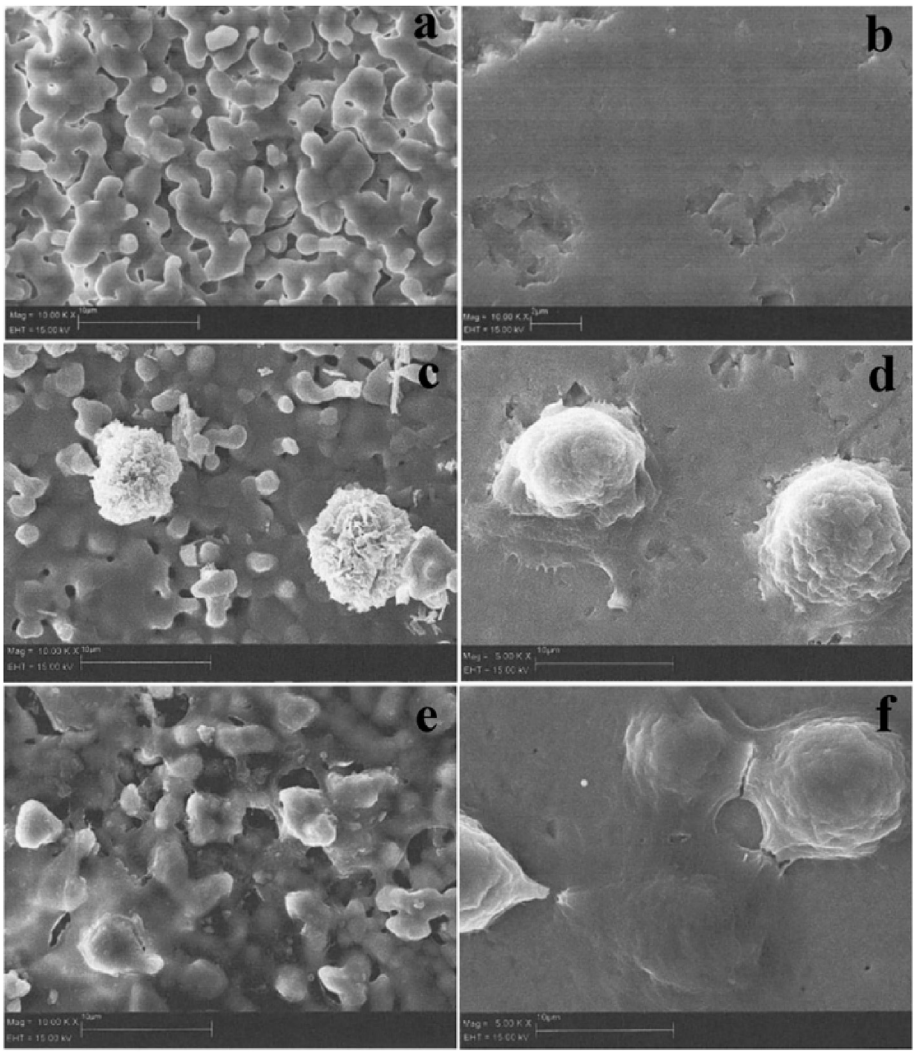

Fig. 5. Scanning electron micrographs: material surfaces of microporous hydroxyapatite (mHA) (a), nonmicroporous hydroxyapatite (pHA) (b); Saos-2 osteoblastic cells cultured for 30 min on mHA (c), pHA (d); Saos-2 osteoblastic cells cultured for 4 h on mHA (e), pHA (f) [49].

the adsorption of endogenous proteins, such as BMP, which is critical through the process of the osteogenic differentiation of stem cells. This result has also been seen in some previous documents. For instance, the micropores in macroporous surface can significantly increase the specific surface area, which improve the adsorption of protein [57]. With the more microporosity, larger specific surface area would boost the ion-exchange, while the dissolution and precipitation process of bone-like apatitic crystals can be positively affected as well [58]. Together, the endogenous protein and transforming growth factors, which play an indispensable part in osteogenic differentiation, can be adsorbed more effectively with more space and larger surface area provided by microporosity.

Moreover, 300 nm thickness bioceramic coatings presented an intermediate value in ALP assessment, which indicate the cell osteoblastic differentiation, compared to microscale topographic and flat surfaces [59]. In the research of Lv et al. [60], 70 nm nanotubes exhibited more obvious osteogenic advantages than 50 nm, 100 nm surfaces and the control group. After hMSCs cultured with or without 7 days and 14 days of osteoinduction, osteogenic-genes Runx2 and osteocalcin were detected in all of the nanotopographic groups. And 70 nm specimens showed the highest gene expression of osteogenic-related genes. The Hench group [28] investigated cell response to different heat treatment, suggesting that the cell behavior responses are not results of difference in surface topography. It seems worthy of note that cell behaviors response to the cooperation of physical and chemical characters of materials.

5. Cell signal transduction in nanoceramic-mediated osteogenesis

Chemical factors, surface roughness and topography collaboratively influence osteoblastic differentiation. Although effects of certain nanoceramic on cell osteoinduction have been reported, the mechanisms of osteogenic differentiation mediated by nanoceramic are not clearly understood. In addition, mechanical loading acts either alone or together with hormones such as parathyroid hormone and oestrogen, stimulating bone formation by changing gene expression. Loading, growth factors and osteocytes released nitric oxide (NO) can activate osteoblasts in direct or indirect ways [61].

5.1. Mechanotransduction

Mechanical force is one of the induce factors in cell mutation which relates to homeostasis and many diseases. The fact that

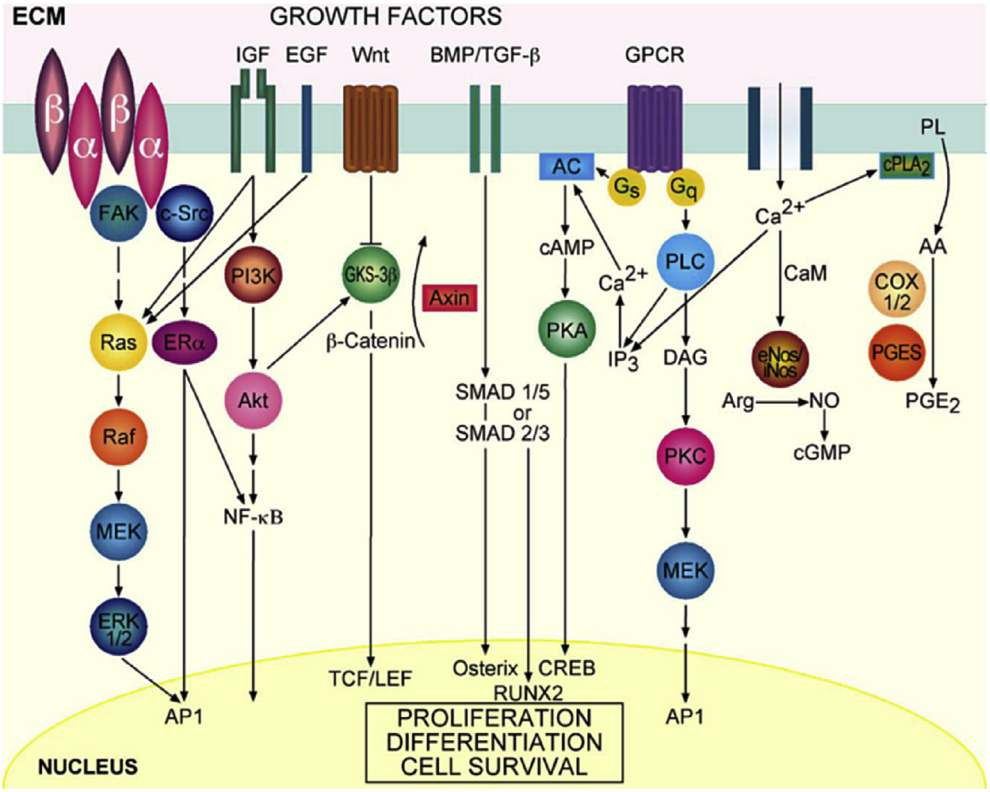

Fig. 6. Mechanotransduction pathways shown to be involved in the mechanical response of osteoblastic cells. Abbreviations: AA, arachidonic acid; AC, adenylate cyclase; Akt, akutely transforming (protein kinase B, serin/threonine kinase); AP1, activator protein 1; BMP, bone morphogenetic protein; CAM, calmodulin; COX1/2, cyclooxygenase 1/2; CREB, c-AMP response element-binding protein; c-Src, tyrosine protein kinase; DAG, diacylglycerol; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ERa, estrogen receptor a; ERK1/2, extracellular signal regulated protein kinase 1/2; FAK, focal adhesion kinase; GSK-3b glycogen synthase kinase-3b; Gs, stimulatory G-protein; GPCR, seven-transmembrane-domain G-proteincoupled receptor; Gq, protein with aq subunit activates PLC, phospholipase C-b; IGF, insulin-like growth factor; iNOS, inducible nitric oxide synthase; IP3, inositol trisphosphate; LEF, lymphoid enhancer-binding factor; MEK, mitogen-activated protein kinase extracellular signal regulated protein kinase (mitogen-activated kinase kinase); NF-jB, nuclear factor-jB; PI3K, phosphoinositide 3-kinase; PGE2, prostaglandin E2; PGES, prostaglandin synthase; PKA, protein kinase A; PKC, protein kinase C; PL, phospholipid; Raf, rat fibrosarcoma serin/threonine protein kinase; SMAD, from sma (small) in Caenorhabditis and mad (mother against decapentaplegic) in Drosophila; Ras, rat sarcoma monomeric GTP-binding protein; TCF, T-cell factor; TGF-b transforming growth factor-b; wnt, from wingless in Drosophila; int, (integration)-1 in mouse [77].

many types of cell naturally function within a complex nanotopographical environment has attracted many researchers to study the cellular response toward the mechanotransduction which was nanotopographically mediated [62]. These studies indicated that most cells react significantly to nanotopographical cues *in vitro*

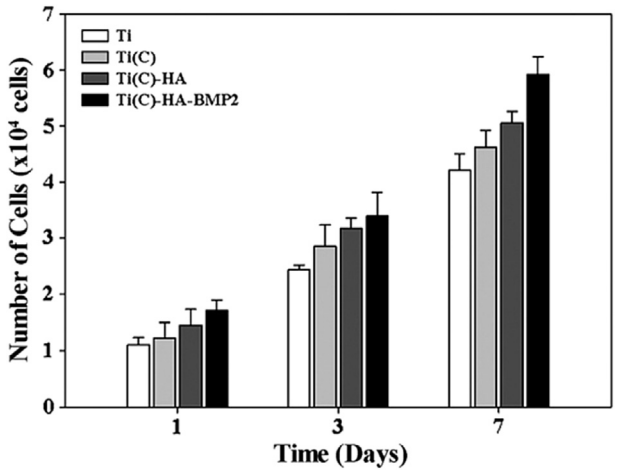

Fig. 7. Mouse osteoblast cells proliferation on the different Ti substrates. As the number of cells was increasing with time, they were more proliferative on the Ti(C)-HA-BMP-2 than those on the other substrates [97].

[62]. The interaction between stem cells and the targeting nanomaterial scaffolds was governed by the molecular mechanisms which namely the mechanotransduction. Physical mechanical stimulation was converted into biochemical signals that then were integrated into the cellular responses [63]. Although the mechanotransdution mechanisms are abstruse, they have greatly intrigued scientists. Jameel Iqbal et al. [64] reported that the mechanotransduction has the similar mechanisms in different cell types. For example, in the recent research by Nikukar et al., mechanical stimulation by piezo ceramic actuators and aluminium reinforcement along with laser interferometry that can produce a peak force of nN magnitude at frequencies of 500 and 1000 Hz could not only change the morphology of both mouse endothelial (Le2) and hMSCs, but also affect the nuclear size of hMSCs [65].

Integrins were widely considered to be used as initiators in mechanotransduction pathway. They constitute a family of cell adhesion molecules [66] and are always presented as heterogeneous dimers comprising of one α and one β subunit. There are 18 α-subunits and 8 β-subunits in mammals. Each αβ arrangement has its unique signaling properties and adhering specificity. In osteoblasts the β1 subunit has the paramount functional role, dimerized with α subunits including α1 through α5 and αV. Moreover, β1 could also integrate with β3 and CD44 [67]. When integrins form bonds with ECM proteins such as fibronectin or vitronectin, in the cell membrane, the reaction between them constitutes a basal pathway for mechanical transmission and generates a signal to

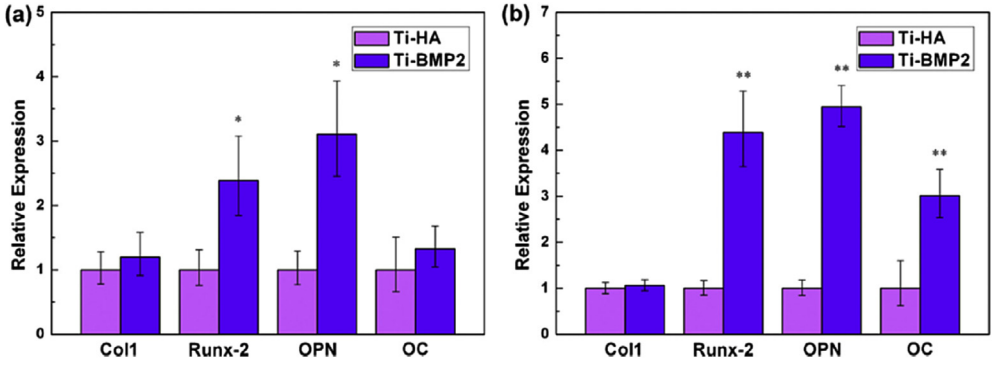

Fig. 8. Relative gene expressions on Ti-HA and Ti-BMP2 after 1 (a) and 2 weeks (b). *, Runx-2 and OPN on Ti-BMP2 is significantly higher than those on Ti-HA after 1 week ($p < 0.05$, $X \pm SD$, $n = 3$); **, much stronger relative expressions of Runx-2, OPN and OC are measured on Ti-BMP2 than those on Ti-HA after 2 weeks ($p < 0.05$, $X \pm SD$, $n = 3$) [99].

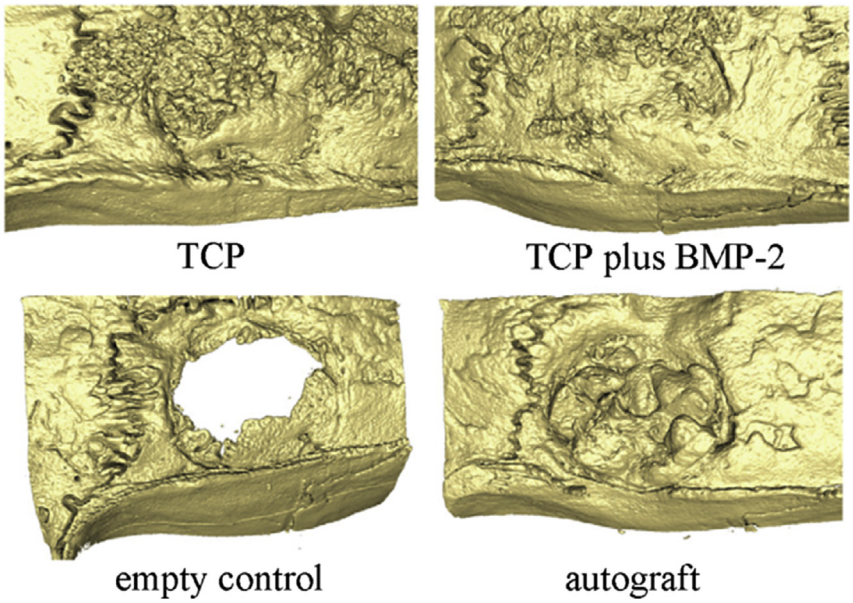

Fig. 9. μCT images of treated and untreated rat calvaria defects: Calvaria defects at day 45 post-surgery were scanned usingμCT. Representative images indicate that complete defect closure was achieved in the two TCP groups and in the autograft group [103].

induce the agglomeration of the actin filaments. The cytoplasmic tail of the β subunit played a critical role in integrin signaling [68] and it interacted with several actin-binding proteins such as talin [69]. Integrins provided a platform for intracellular signaling, but they have exhibited non-enzymatic activity in their cytoplasmic domains [70]. Downstream signaling pathways, mediated by non-receptor tyrosine kinase [71], was used to induce the process of extracellular mechanical signals to convert into functional reaction by integrins [72]. A primal downstream anchor is the extracellular signal regulated kinase (ERK), which is a member of the mitogen activated protein kinase (MAPK). The ERK/MAPK pathway is one regulator of the proliferation and differentiation of stem cells. ERK-MAPK signaling conveyed physical information from the extracellular environment to the nucleus [70] and regulated the cell cycle. Furthermore, except for affecting both replication and cyclicality of cells, the ERK/MAPK pathway also has been involved in the differential response of bone cells to a variety of signals, such as ECM-integrin binding [72], and mechanical loading [73]. A central target in integrin-mediated signaling is focal adhesion kinase (FAK), which was considered to be positive to the link between cell surface integrin-ECM binding and activation of ERK [74]. The activated FAK roused extra intermediators of cytoskeletal tension: RhoA (a small GTPase protein) and its effector ROCK [72,75], which were known to regulate actin cytoskeleton and focal adhesions by inducing the formation of stress fibers. Shi et al. [76] reported that the RhoA/ROCK pathway also affect the proliferation and differentiation in stem cells by activate ERK/MAPK pathway.

In addition to the signaling pathways mentioned above, other research groups have investigated pathways in mechano-transduction. Receptors in the mechanical signaling transduction pathway, such as cadherins and stretch-spreading activated calcium ion (Ca^{2+}) channels, transmitted diverse signals. These various mechanical signal pathways could do a synergic work or exert an independent effect on the mechanotransduction process. Subsequently, the gene expression in cell nucleus is regulated by them [77]. It is suggested that the mechanotransduction is mediated by endocrines, the extracellular matrix (ECM) and the modality of the mechanical stimulations. Paul et al. [78] have reported that LDL-receptor-related protein 5 (LRP5) [79,80], the Wnt binding receptor, is a key protein that connect with the loss-of-function mutations of bone. Ingredients of the Wnt signaling pathway facilitated the mechanotransduction result in generating the available bone components [78]. Moreover, compounds THQ-1a and PP-9 modulated Wnt signaling and highly enhanced the

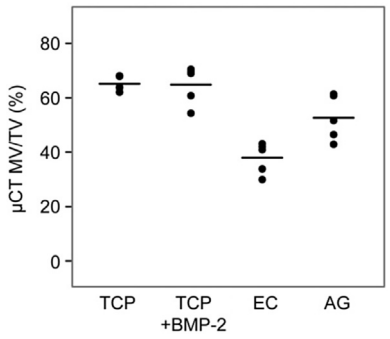

Fig. 10. Quantitative μCT analysis of total mineralized tissue in rat calvaria defects. EC: empty control; AG: autograft [103].

expression of molecular marker such as osteocalcin and collagenI in C2C12 cell osteoinduction [81]. After loading, the Lrp5 acted as an intermediator in adult osteoblast operation [82]. The fluid flow is an elementary mechanical irritant in bone lacunar–canalicular system which is composed with steady flow and turbulent flow Lu et al. [83] have discovered that the process of calcium ion response to the skeleton networks is evidently depend on the characters of fluid flow. Fig. 6 showed the process of mechanotransduction in stem cells [77]. The interaction between nanomaterials and stem cells is complex, but the mechanotransduction can be a crucial regulative control of differentiation and proliferation in stem cells.

5.2. Roles of calcium ion in osteoinductions

Ion channels were reported to significantly regulate osteogenesis of MC3T3-E1. Jung et al. proposed that extracellular Ca^{2+} which derived from HAP dissolution, might be internalized through L-type calcium channel and active the CaMK2α/CAM pathway [84]. And cell osteogenesis was mediated by Ca^{2+} from both the involvement of L-type and non-L-type calcium channels and calcium sensing receptors (CaSR). CaMK2α/CAM pathway eventually modulate osteogenic differentiation through the c-AMP response element-binding protein (CREB) or the extracellular signal regulated protein kinase 1/2 (ERK1/2) pathway [85].

6. Role of carrier

Bone morphogenetic proteins (BMPs) are versatile natural osteoinductive growth factors, which are ingredients of the transforming growth factor β (TGF-β) superfamily. Several *in vitro* and *in vivo* [86–90] researches showed that BMPs play positive roles in formation of both bone and cartilage. Currently, 15 BMPs have been identified already [91]. Among them, BMP-2 and BMP-7 have been investigated to participate in a variety of biological process, such as the proliferation, differentiation and migration of cells, and osteogenic differentiation which lead to the regeneration of new bone [92,93]. BMP-2 [94] and BMP-7 [95] have been approved by FDA to use in clinical therapy [90]. A lot of experiments have been done to study the combination of BMPs and carrier such as calcium phosphate (CaP) ceramics and collagens. Collagens derived from animal body used as carriers have disadvantages of causing antigen-antibody reaction and spreading diseases, as well as lacking suitable support mechanical strength when used as the scaffolds [96]. CaP ceramic coatings are widely used as carriers to adsorb BMPs in orthopedic surgery researches.

Nanoscaled HA was one kind of considered potential carrier in therapeutic delivery system. In this system, BMPs play the osteoinductive role and HA show the effect of bone conductive. Both of these two factors have equal effects in the process of new bone formation and bone regeneration. A recent research [97] have studied the differences of mouse osteoblast cells seeded on different hydroxyapatite-formed Ti surface. The authors immersed the surface pretreated Ti discs in a simulated body fluid (SBF) solution to get a Ti surface with HA crystals. As the carrier, HA need to be treated to have chemical binds with BMP-2. After coating BMP-2 on Ti-HA surface, a series of relevant tests have been done to test the cell differentiation and proliferation properties of BMP-2 combined Ti-HA (shown in Fig. 7), and they observed a quicker cell proliferation on Ti-HA-BMP-2 group that other group without being treated [97]. In another research [98], researchers made a HA coating on surface of Ti6Al4V substrates by plasma spaying method after the substrates pretreated by being gritblasted. Then cultured BMP-2 gene modified and non BMP-2 gene modified rat bone marrow mesenchymal stem cells (rBMSCs) onto HA coated substrates, respectively. The results showed that, the first mentioned of the two have a higher osteogenic proteins expression in osteogenic differentiation process (shown in Fig. 8). Human osteosarcoma MG63 cells (ATCC) cultured onto the similar Ti alloy substrates which coating with BMP-2 conjugated hydroxyapatite have the same results [99]. Porous HA was used to form a three dimension scaffold after mixed with hydrogels and BMP-2 [100]. In animal experiment, this combinatorial material boosted the efficiency of MSCs differentiation *in vivo*. In addition, TCP was used in BMPs delivery system as well. TCP/HAP porous ceramics granules were coated with polyelectrolyte multilayer films (PEM), which are able to avoid albuminous degeneration, to deliver rhBMP-2 [101]. The results showed that TCP/HAP loaded with rhBMP-2 exhibited both bone conductive and osteoinductive properties well. Medical grade poly ε-caprolactone/tricalcium phosphate/collagen (mPCL/TCP/collagen) scaffolds loaded rhBMP-2 implant into a rat calvarial defect [102]. Compared to non-rhBMP-2 loaded scaffolds, the treated groups showed complete defect sites healing by 15 weeks *in vivo*. Interestingly, on the contrary, a recent research processed in rat calvaria defects sites investigated whether the bone conductive properties of β-TCP can be enhanced when loading BMP-2, and all the histomorphometric analysis results showed that TCP can prompt the maximal bone formation without the presence of BMP-2 (shown in Figs. 9 and 10) [103].

Compared with BMP-2, there are fewer articles about combination of BMP-7, which is also known as OP-1, with HA or TCP. Macroporous HA scaffolds were fabricated by hydrothermal chemical exchange method to load with hOP-1 [104] in evaluating the induction of osteogenic proteins in bone formation. In another research [103], HA scaffolds with a porous rate of 74.6% loaded with BMP-7 were investigated to have significant increase in enhancement of osteoinduction when hMSCs cultured on *in vitro*. Morgan et al. [105] used TCPs as carriers to load BMP-7 in metaphyseal bone healing. Biphasic calcium phosphate (BCP) ceramic scaffolds with microporous surfaces were used as carriers in the combination of BMP-7 with VEGF or MSCs [106]. The results showed that the effect of these combination on bone formation is not obvious compared with the controlled group, whereas, BMP-7 with the present BCP ceramic scaffolds revealed bone induction. Based on these discussed researches, CaP ceramic coatings used as carriers of BMPs could play positive roles in osteoinduction of bone formation.

7. Conclusion

This review summarized the effects of some physical and chemical properties of nanoscaled ceramic coatings constructed on Ti-based alloys on the stem cells behaviors, which can be summarized in two aspects: protein adsorption and cell adhesion. The surface physical characteristics could influence the behaviors of stem cells through the modification of scaffold topography and

roughness. Ceramic coatings with smaller feature sizes, including R_a, grain size and particle size, may adsorb more proteins than that with larger feature sizes. But its effect on cell adhesion is still in dispute and needs further investigation. Micropores provide more binding sites and larger surface areas for protein adsorption and cell adhesion. Besides these factors, the coatings stiffness maybe also influence the cell behaviors. It has been reported that the substrate stiffness, moderate, rigid matrices, and soft can promote the differentiation of MSCs into neuronal-like cells, osteogenic differentiation, and myogenic differentiation, respectively [107]. The chemical way to modulate the cell behaviors is mainly carried out by adjusting the chemical compositions of coatings. The contact process between ceramic coatings and stem cells rely on mechanotransduction pathways. Mechanical forces induce the mutation of cells. But the mechanotransduction pathways are not completely understood yet. For being used as carriers of BMPs, ceramic coatings are studied to play osteoinductive and osteoconductive roles in new bone formation *in vivo* and *in vitro*. Therefore, further investigations about nanoceramic coatings could be made in mechanotransduction pathways and carry BMPs.

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