The effect of nano-scale topography on osteogenic differentiation of mesenchymal stem cells

Faezeh Faghihia,b, Mohamadreza Baghaban Eslaminejada

Background. Large bone defects resulting from trauma or disease pose a threat to humans. Thus far, tissue engineering as an important clinical approach uses cells, growth factors and scaffolds to regenerate large areas of damaged bone tissue. Since bone is a nanocomposite structure, it is assumed that nanomaterial scaffolds can induce or promote osteogenesis by mimicking the cell niche at nano level.

Methods and Results. In this review we highlighted the effect of nano-scale topography on osteogenic differentiation of Mesenchymal Stem Cells (MSCs) as potent cell candidates in bone engineered constructs. The key point in the induction of differentiation by nanomaterials is the discontinuity in their topography. This leads to alteration in protein adsorption and restriction of extracellular matrix deposition by the cells and consequently leads to changes in cell morphology and the frequency of accessible sites for cell adhesion. Here, we have reviewed the literature on the role of different types of nanomaterial scaffolds in osteogenic differentiation of these cells. Since little is known about the underlying molecular networks induced by nanomaterials, we also reviewed possible underlying mechanisms of nanotopographical effects on the osteogenic differentiation of MSCs.

Conclusions. Nano-scale materials provide a niche which is very similar to native bone in geometry and stiffness. Such nano-scale topographies improve the function of MSC-based engineered constructs in regeneration of bone defects.

Key words: mesenchymal stem cells, bone tissue engineering, nano-scale topography, osteogenic differentiation

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INTRODUCTION

Bone, the most common transplanted tissue after blood1, is known to be a supportive nanocomposite structure that consists of soft and hard inorganic components2,3. Hydroxyapatite is the dominant, nanocrystalline component of bone: it has a thickness of 2-5 nanometers4. Proteins, of nanometer size, assemble together to form a nanostructured extracellular matrix (ECM), which in turn influences the adhesion, proliferation, and differentiation of different cell types, such as osteoblasts and mesenchymal stem cells5.

As a dynamic structure, defects due to trauma and disease heal spontaneously, however large defects that can delay or impair healing need additional treatment before they can regenerate. There are two main options in order to reconstruct large bone defects: the application of bone grafts (autograft, allograft, and xenograft) and bone constructs fabricated based on bone tissue engineering principles.

Although bone grafts have been used for decades in the clinic setting, drawbacks such as supply limitation, risk of donor site morbidity (autografts), pathogen transmission and rejection by the recipient’s body (allografts and xenografts) limit their applications in therapy6. Concerns associated with bone graft transplantation have challenged scientists to search for appropriate substitutes. Their attempts have resulted in bone construct manufacturing as based on tissue engineering principles.

The term “tissue engineering” refers to the application of principles and methods of biology and engineering in order to develop functional substitutes for the repair and regeneration of damaged tissues7-9.

This concept is comprised of three building blocks of cells, the matrix (scaffold), and osteoinductive growth factors10. Each of these elements alone can promote tissue regeneration, but constructs fabricated with a combination of all three components are more effective.

In order to manufacture a well-elaborated bone construct, it is beneficial to mimic the in vivo 3D niche for osteoprogenitor cells inside the scaffold by exposing them to appropriate chemical and physical stimuli11, similar to natural bone.

Most fabricated bone grafts usually mimic bone structure and topography at the micro-level; however today, researchers are focusing on designing bone constructs with appropriate biomechanical properties and biomimetic behaviors at the nanolevel12. In order for the constructs to promote both osteogenic differentiation and allow for function of the osteoprogenitor cells (such as MSCs) in fabricated bones.
In this review, we will provide an overview of the topographic effects of nanomaterials on the osteogenic differentiation of MSC-based bone constructs.

**MESENCHYMAL STEM CELLS: POTENT CELL CANDIDATES IN BONE TISSUE ENGINEERING**

Mesenchymal stem cells (MSCs): Definition and characterization

MSCs or colony forming unit-fibroblast like cells, were discovered by Friedenstein in the 1970s. These cells express many surface antigens including: STRO-1, CD105, SH3, CD29, CD44, CD71, CD90, CD106, and CD124 (ref.13,14). MSCs can be isolated easily from different sources such as the bone marrow13, adipose tissue15, synovial membrane16, skeletal muscle17, and teeth18. They possess two important characteristics: the potential to self-renew for a relatively long time and the ability to differentiate along multiple cell lineages, including osteoblasts, chondrocytes, adipocytes, and myocytes13 (Table 1).

An important consideration of MSCs is that they can be a good alternative to other types of cells for bone repair and regeneration21-31. Gentleman and his colleagues have shown that bone nodules formed by MSCs are very similar to native bone nodules in terms of stiffness and nanoarchitecture, when compared with nodules formed by other cell types such as embryonic stem cells32.

**Osteogenic differentiation property of MSCs**

The osteogenic differentiation property of MSCs is a complex process that involves various environmental factors such as hormones and growth factors33-40. Chemicals such as Lithium chloride41, 1,25-dihydroxyvitamin D3 (ref.42), dexamethasone, ascorbic acid, and beta glycerol phosphates are also known inducers of osteogenesis in mesenchymal stem cells42-44 (Table 2).

In the absence of dexamethasone no differentiation occurs in human MSC cultures45. Ascorbic acid, on the other hand, enriches the deposition of matrix with collagen64, while beta-glycerol phosphate, as a phosphate enriched organic compound, has a role in matrix mineralization67.

Each osteogenic factor exerts its effect through a distinct signaling pathway, of which some are not well known (dexamethasone), whereas other pathways are recognized to a certain degree for example, BMPs mediate their

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**Table 1.** Multiple sources of mesenchymal stem cells, their characterization and multilineage differentiation capacity.

<table>
<thead>
<tr>
<th>Sources of MSCs</th>
<th>bone marrow, adipose tissue, synovial membrane, skeletal muscle, cord blood19</th>
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<tr>
<th>Characterization of MSCs</th>
<th>Positively express: CD73, CD105, and CD90</th>
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<tbody>
<tr>
<td></td>
<td>Negatively express: CD34, CD45, CD14, CD11b, CD19, CD79a, HLA-DR</td>
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<td></td>
<td>Osteogenic, Chondrogenic as well as Adipogenic differentiation capacity20</td>
</tr>
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| Multilineage differentiation capacity | osteocytes, chondrocytes, adipocytes15, skeletal muscle cells21, cardiac muscle22, smooth muscle cells23, neural cells24, hepatocytes25, endothelial cells26 |

**Table 2.** The list of frequently used hormones, growth factors and small molecules which induce osteogenic differentiation in mesenchymal stem cells.

<table>
<thead>
<tr>
<th>Osteoinductive Substances</th>
<th>References</th>
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<tbody>
<tr>
<td>Prostaglandin E2</td>
<td>Scutt et al.33, Ninomiya et al.45</td>
</tr>
<tr>
<td>Sonic hedgehog</td>
<td>Spinella et al.14, James et al.46, Cai et al.47</td>
</tr>
<tr>
<td>Insulin-like growth factor-1</td>
<td>Koach et al.35</td>
</tr>
<tr>
<td>BMP-2</td>
<td>Gori et al.38, Jorgensen et al.41, Pick et al.44, Boehm et al.46, Feng et al.50</td>
</tr>
<tr>
<td>BMP-6</td>
<td>Gruber et al.30, Zhu et al.43</td>
</tr>
<tr>
<td>BMP-7</td>
<td>Gruber et al.30, Qing et al.52</td>
</tr>
<tr>
<td>TGF-beta</td>
<td>Ng et al.33, Zhou et al.54</td>
</tr>
<tr>
<td>FGF</td>
<td>Kiyono et al.53</td>
</tr>
<tr>
<td>Platelet Rich Plasma</td>
<td>van den Dolder et al.16, Hu et al.57, Elbackly et al.58</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Wang et al.19, Fiorentini et al.40</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Toai et al.61, Olvares-Navarrete et al.62</td>
</tr>
<tr>
<td>PTHR</td>
<td>Sammons et al.63, Suriyachand et al.64</td>
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osteogenic effects via activation of Smad transcription factors, while parathyroid hormone induces the expression of osteogenic genes via the G-protein coupled receptor signaling pathway. The activation of these signaling molecules by hormones or growth factors leads to the expression of RUNX-2, a pivotal and early transcription factor involved in osteogenesis (Fig. 1).

**Clinical advantages of MSCs in bone regeneration**

Besides its well-osteogenic differentiation capacity and easy access from different tissues, the immunomodulatory property of MSCs is a key issue in the field of regenerative medicine. The ability of MSCs to modulate immune responses makes it feasible to use them in allogenic transplantsations without any substantial risk for immune rejection. Evidence exists regarding the ability of MSCs to inhibit the proliferation of T-cells and B-cells. Moreover, MSCs can suppress the proliferation of natural killer cells, cytokine secretion and cytotoxic properties, in addition to inhibiting dendritic cell maturation and activation. An intermediate level of expression for MHC class I along with a very low level of expression in MHC class II has increased medical interest in using MSCs as an appropriate cell source for clinical application in the field of bone regenerative medicine.

**MESENCHYMAL STEM CELLS AND PHYSICAL CUES FROM THE ECM**

In addition to the chemicals that have osteoinductive effects on the differentiation fate of MSCs, the biomimetic nanostructured substrate where MSCs are located plays a prominent role in the osteogenic differentiation of these types of cells.

There is a common consensus among scientists that each type of stem cell exists in a tissue specific microenvironment called the niche, and that the fate of the stem cell relies on the properties of this niche. Tissue specific structural components, biomechanical forces, and the gradients of cytokines that are provided or supported by the ECM around the cells enable them to have a good “sense of touch.” This 3D system supports cell-to-cell interaction, cell migration and division, and cell differentiation via two types of signals: physical and chemical.

Chemical cues include growth factors, biomolecules, or any type of functional groups that bind to cell membrane receptors to support cell proliferation or differentiation. Physical cues, which are presumed to have a marked role in the differentiation of MSCs, are comprised of three subgroups: mechanical stimuli, electromagnetic, and topographical. Recent studies have demonstrated that mechanical properties of the extracellular environment trigger cell structure and function and play a pivotal role in the regulation of tissue architecture and organization. There are a large number of reports on osteogenic differentiation that can result from mechanical stimuli, including compression and fluid shear stress. Shear stress from the activation of mechanosensitive ion channels, Ca²⁺ channels, heterotrimeric G-proteins, and protein kinases enhances matrix mineralization and the expression of osteoblastic genes in human MSCs. Besides mechanical forces, electromagnetic cues also enhance the osteoblastic differentiation of MSCs. Recent studies on the application of electrical stimulation on MSC-based constructs have caused us to consider this approach more in clinical applications.

Keeping these points in mind, our focus of interest is on the topographic effects of nano-scale biomaterials (e.g., pits and grooves) with regards to the osteogenic differentiation of MSCs.
**Topographical cues**

As mentioned earlier, the scaffold (as an artificial ECM) plays a pivotal role in the concept of tissue engineering. Cells that lie inside the scaffold undergo differentiation while secreting a new native ECM, which is essential for tissue regeneration. Thus the important issue in the fabrication of an artificial ECM (scaffold) is that it should be designed with maximum resemblance to the native microenvironment.

Due to the existence of various types of collagen nanofibers and nanocrystalline hydroxyapatite (HAp) in the bone, native ECM is comprised of a complex mixture of pores and ridges of a nanometer scale diameter. Decrease in the size of substrate materials to nanoscale level, leads to increase in surface area, the ratio of the surface to the volume, and surface roughness. This phenomenon consequently qualifies surface properties in the transmission of cell matrix signaling, cell activity, cell morphology, adhesion, motility, and proliferation, as well as gene regulation. Based on Laurencin’s study, cells attach better to fibers that have diameters smaller than their own. Therefore, if the cells are seeded on components of equal or greater cell diameters, there would be no normal behavior and the expression of phenotypic markers for stimulation of cell growth and tissue regeneration.

**MSCs on nanoscale materials**

Any type of particle, tube, or fiber that is created from metals, ceramics, polymers, or composites smaller than 100 nm in at least one dimension is called a nanomaterial. Research has shown that the surface properties of nanomaterials such as chemical characteristics, stiffness, and nanotopography, in particular pits and grooves, have a tremendous impact on cell attachment and differentiation. Although only a few papers have been written about the topographic effects of cell attachment and proliferation, it is generally accepted that nanotopography affects cell properties. Nanogratings enhance adhesion but reduce the rate of proliferation of adhesive cells in comparison with planar substrates, while nanopits reduce cell adhesion but reduce the rate of proliferation of adhesive cells in comparison with planar substrates. Nearly all types of cells, and MSCs in particular, align along the long axis of the grooves on substrates, while nanopits and nanoposts generally reduce cell attachment. Nearly all types of cells, and MSCs in particular, align along the long axis of the grooves on substrates.

**Osteogenic differentiation of MSCs on nanomaterials**

Nanomaterials can be appropriate biocompatible substitutes for the regeneration of bone defects. It is now well documented that there is an increase in cell growth and osteodifferentiation in 3D nanostructured scaffolds compared to smooth 3D substrates. Different types of biomaterials in the form of ceramics, polymers, or composites are fabricated at the nanolevel in order for their applications in regenerative bone medicine.

In order to evaluate the effect of nanotopographic geometry on human MSC osteogenic differentiation, Dalby and colleagues have shown that random circular nanostructures promote and direct osteoblast differentiation of MSCs without any need for osteogenic promotion of the cell culture medium. By culturing Stro-1-enriched human MSCs in polymethylmetacrylate with varying degrees of disorder and geometry they found that MSCs differentially responded to substrate properties. Interestingly, the symmetry and order of nanopits on the topography of the scaffolds were important for the expression of specific osteogenic proteins, including osteocalcin and osteopontin.

Nanophase ceramics are also seen as appropriate bone substitutes due to their ability to promote mineralization. In our study, we fabricated a novel HAp/gelatin scaffold coated with nano-HAp in a nano-bed configuration with the intent to evaluate the effect of nano-HAp coatings on the biocompatibility of the scaffold in response to MSCs. Our results show that the incorporation of rod-like nano-HAp and the coating of scaffolds with nano-HAp particles enabled the prepared scaffolds to possess the desirable biocompatibility, high bioactivity, and sufficient mechanical strength compared to non-coated HAp samples. Ceramics, alone or in combination with polymers, have been shown to be capable of inserting their osteoinductive properties in a composite cassette. Polini and colleagues described a nanofibrous composite, including poly-caprolacton (PCL) and nano-HAp or beta-tricalcium phosphate (TCP). Their study has confirmed that mineral nanophase structurally regulates the osteogenic differentiation of human MSCs in the absence of any osteoinductive chemicals. Application of PCL in combination with Collagen and HAp by Phipps et al. could increase the expression of signaling molecules involved in cell survival and osteoblastic differentiation.

Our group has also researched the effect of the nano-hydroxyapatite/poly-(lactic acid) composite, with different morphologies on bone differentiation of MSCs. Our results have shown that needle-like nano-hydroxyapatite/poly-(lactic acid) composite provides the most appropriate matrix for producing bone constructs using MSCs.

In another study, Lee and colleagues created fibrous scaffolds with poly lactide-co-glycolide (PLGA) and nano-sized hydroxyapatite using the electrospinning method. The administration of HAp on these nanofibers had no adverse effect on cell viability, but it also increased ALP activity, calcium mineralization, and expression of osteogenic genes. Lock et al., also showed that Nano-
those cells that were only cultured on the genipin-chitosan framework\textsuperscript{125}.

Not only ceramics, but also other nano-treated surfaces have been shown to have a direct effect on cell growth and osteogenic differentiation. The TiO\textsubscript{2}–nano network on the Ti surface has been shown by Chiang et al. to promote human MSC growth and osteogenic differentiation, when compared to an untreated Ti surface without the TiO\textsubscript{2} nano network\textsuperscript{142}. Another group documented the effect of the Titanium-hydroxyapatite nanocomposite coating (grain size <50 nm) on human MSCs cytoskeleton organization, cell matrix adhesion, and mineralization. Interestingly, TiO\textsubscript{2}-HAp coating surface property was able to induce osteogenic differentiation of human MSCs in the absence of chemical treatments\textsuperscript{143}.

Besides nanohydroxyapatite particles, nanofibers and nanotubes of different composition could enhance biomineralization when compared to solid-walled scaffolds\textsuperscript{144}. Hosseinkhani et al. in 2006 have shown that a 3D network of nanofibers formed by the self-assembly of peptide amphiphile molecules may increase proliferation and differentiation of MSCs compared to the static culture system\textsuperscript{145} solely by altering carbon nanotube dimensions. Oh and colleagues have been able to direct stem cell differentiation towards osteogenic lineages. They found that by increasing the diameter of nanotubes from 30 nm to 100 nm the adhesion, elongation, and differentiation of stem cells would change. It was shown that at 30 nm diameter, the cells exhibited a round morphology with a high level of adhesion; at 100 nm diameter, cells showed an elongated morphology with a low level of adhesion, but with a high potential to differentiate into an osteogenic lineage when compared with cells in 30 nm diameter tubes.

**THE UNDERLYING MECHANISMS OF NATTOPHYSICAL EFFECTS ON THE OSTEOGENIC DIFFERENTIATION OF MSCS**

The ability of nanotopographical cues to control osteogenic differentiation in MSCs has attracted the attention of scientists to understand underlying biological mechanisms; however, despite the large application of nanomaterials in bone tissue engineering, little is known about the corresponding molecular networks. The key point in the induction of differentiation by nanomaterials is the discontinuity in their topography. This leads to alteration in protein adsorption and restriction of ECM deposition by the cells. This property consequently leads to changes in cell morphology\textsuperscript{146} and the frequency of accessible sites for cell adhesion\textsuperscript{147}.

One of the key structures that manage the interaction between the cell and topography of the substrate are focal adhesions: FAs (ref.\textsuperscript{147,148}). Cells attach to their environments via FAs that are 15-30 nm in diameter\textsuperscript{149}. These nanodynamic clusters are enriched in integrins and cytoskeletal signaling proteins, including talin, alpha-actinin, and focal adhesion kinases (FAKs). Fabry and colleagues have highlighted the role of FAKs in cell...
behavior by showing that FAK deficient cells have lower cell stiffness, reduced adhesion strength, and increased cytoskeletal dynamics compared to wild-type cells\textsuperscript{150}. As signaling organelles, FA sites enable cells to touch their environment by transmitting mechanical and physical signals from the matrix to the cell\textsuperscript{105,147,151} (Fig. 3).

From the biological point of view, most normal cell types depend on physical cues from their surrounding matrix in order to respond efficiently to growth factors\textsuperscript{154}. In order to probe their stability on the substrate, cells continuously apply small “cytoskeletal traction forces” on FA sites\textsuperscript{155}. If opposing external force (from the matrix) and internal physical forces (from the cell) are unequal, cell surface adhesion clusters will start moving to achieve to a stable position\textsuperscript{156}. There are a large number of studies showing that nanotopographical cues modulate integrin clustering and the formation of FAs (ref.\textsuperscript{157-159}). Any changes in their density are linked to changes in stem cell differentiation\textsuperscript{160-162}. Based on research by Hart and colleagues in 2007, the functional differentiation of osteoprogenitor cells is highly regulated by formation of FAs and cell processes due to nanotopographical cues\textsuperscript{163}. By quantifying the adhesion rate of primary osteoblasts on nanoscale substrates, Biggs and colleagues have concluded that nanomaterial features influence differential networks by regulating the numbers of integrin clustering and formation of focal adhesions\textsuperscript{158}. Nanotopographic features may lead to changes in the number and arrangements of FAs (ref.\textsuperscript{164}). This asymmetric distribution then transmits signals to the cell via connections between FAs and cyto-nucleoskeletal proteins\textsuperscript{114}. In other words, these cell-matrix adhesion sites (FAs) transduce mechanical stress into chemical signals inside the cells\textsuperscript{165}. There is a correlation between the number of FAs and the density of matrix proteins that contain the RGD motif\textsuperscript{166}. The beta-integrin subunit of FA is associated with FAK, which is a non-receptor tyrosine kinase\textsuperscript{167}. This enzyme influences the cell transcriptome profile and regulates stem cell differentiation\textsuperscript{168} via the adhesion-dependent phosphorylation of downstream protein kinases such as extracellular signal regulated kinase: ERK (ref.\textsuperscript{169,170}). It seems that transferring ERK 1/2 from cytoplasm to the nucleus is the principle event in the modulation of differentiation\textsuperscript{157}, control of cell proliferation\textsuperscript{171,172}, and expression of corresponding transcription factors. There are studies indicating that an increase in the number of FAs, and subsequent FAK activation, causes induction of osteogenesis versus adipogenesis in MSCs (ref.\textsuperscript{173,177}). Shih et al. showed that activities of FAK and ERK1/2 kinases increase on stiffer substrates during osteogenic differentiation\textsuperscript{178}. A recent study by Kulangara et al. showed that the expression of a zinc-binding phosphoprotein, Zyxin, depends on “FA remodeling in response to nanotopographical changes”\textsuperscript{179}. Any changes in expression of Zyxin, eventually modulates gene expression and cytoskeletal reorganization\textsuperscript{179}.

The interaction of the cell with the surface of the substrate (topography) may induce the growth of focal contacts in response to phosphorylation of Rho GTPase (ref.\textsuperscript{177,180}). FAs are constructed under the control of Rho GTPase. A dynamic regulation exists between the activity of Rho GTPase and the formation of FA for cell migration\textsuperscript{180,181}. These molecules play a role in FA complex maturation by recruiting actin filaments and integrins. The activation of endogenous Rho controls actin formation and cytoskeleton remodeling in addition to affecting gene expression, cell morphogenesis, cell cycle progression\textsuperscript{185,186}, and consequently the switching on of commitment signaling pathways towards osteoblastic lineages. Small Rho-family GTPases (particularly Rac-1 and RhoA) regulate actin assembly and contraction\textsuperscript{187}. Changes in actin dynamics are monitored by myocardin-related tran-
CONCLUSION AND FUTURE CHALLENGES

As a cell, touching the environment does not only mean to sense the medium and growth factors that have been added to the medium, but also involves the composition and topography of the scaffold where the cell lies. As much as the composition and topography of scaffolds resemble the cell’s native niche, it enables differentiation into the corresponding cell type. The structure and topography of the scaffold at the nanolevel determines the number of adhesion sites of the cell to the scaffold, and indirectly manages the qualification of underlying molecular pathways inside the cell, from the cytoskeleton to the nucleoskeleton. These epigenetic events eventually switch on or off the corresponding genes based on chromosome positioning. Thus nanotopography, including the effects of size, scale and dimension of the substrate plays a prominent role in the decision of a cell’s fate. Nanomaterial science, biology, and medicine are at the beginning of their inter-relationship. By improving manufacturing techniques in the fabrication of materials at a nanolevel, engineers attempt to increase the numbers and quality of scaffolds that can be used in bone engineering. However nanomaterial behavior at the transplantation site, its biocompatibility, cytotoxicity, and biodegradability are the most important criteria in the field of biomedical engineering. This should be determined in vitro and in vivo in animal experiments by either biologists or clinical trials. The manufacturing of new scaffolds at the nanolevel and testing of the biocompatibility and cytotoxicity of the fabricated nanomaterials are the most important issues to be thoroughly studied before their therapeutic applications.

ABBREVIATIONS

Akt (or Protein Kinase B), Ak (mouse strain) t (Thymoma); ALP, Alkaline phosphatase; BMPs, Bone Morphogenetic Proteins; Ca2+ Channels. Calcium ion channels; CD, Cluster of Differentiation; Dlx5, Distal-less homeobox5; Col I, Collagen type I; DNA, Deoxyribonucleic acid; ECM, Extracellular Matrix; ERK, Extracellular Regulated Kinase; ERM, Ezrin, Radixin, and Meosin; FAs, Focal Adhesions; F-Actin, Filamentous Actin; FAK, Focal Adhesion Kinase; FGF, Fibroblast Growth Factor; G-actins, Globular Actins; G-proteins, guanine nucleotide-binding proteins; GSK-3β, Glycogen synthase kinase 3-beta; GTP, guanosine triphosphate; HAP, hydroxyapatite; LIMK. (Lin11/Isll/Mec3) Kinase; MHC, Major Histocompatibility Complex; MRTF, Myosin-Related Transcription Factor; MSCs, Mesenchymal Stem Cells; Msx2, Mash homeobox homologue 2. mTORC2, Mammalian Target of Rapamycin Complex 2; OPN, Osteopontin; OSC, Osteocalcin; P120 or Catenin Delta 1, A prototypic member of Armadillo protein family; Pa, Pascal; PCL, Poly-Caprolactone; PO4, A Phosphate group; PLGA, Poly lactide-co-glycolide; PLLA, Poly-L-lactide; PTHR, Parathyroid Hormone
Receptor; P2X5, purinergic receptor P2X, ligand-gated ion channel, 5; P2Y receptors, Family of purinergic receptors stimulated by nucleotides; ROCK, Rho-associated protein kinase; RUNX-2, Runt-related transcription factor-2; Smad, from gene SMA for small body size and abnormal protein kinase; RUNX-2, Runt-related transcription factor; TiO2, Titanium Oxide.

CONFLICT OF INTEREST STATEMENT

The authors stated that there is no conflict of interest regarding the publication of this article.

REFERENCES

37. Gruber R, Kandler B, Fuerst G, Fischer MB, Watzek G. Porcine sinus mucosa holds cells that respond to bone morphogenetic protein


49. Lee MH, Kwon TG, Park HS, Wozney JM, Ryoo HM, BMP-2-induced Osterix expression is mediated by Dlk1 but is independent of Runx2. Biochemical and Biophysical Research Communications 2003;309(3):689-94.


139. Lee JH, Rim NG, Jung HS, Shin H. Control of osteogenic differentiation and mineralization of human mesenchymal stem cells on composite nanofibers containing poly(lactic-co-glycolic acid) and hydroxyapatite. Macromol Biosci 2010;10(2):173-82.


168. Salasnyk RM, Klees RF, Boskey A, Plopper GE. Activation of FAK is necessary for the osteogenic differentiation of human mesenchymal stem cells on laminin-5. Journal of Cellular biochemistry 2007;100(2):499-514


175. Li J, Xie D. Cleavage of focal adhesion kinase (FAK) is essential in adipocyte differentiation. Biochemical and Biophysical Research Communications 2007;357(3):648-54.


192. Haque F, Lloyd DJ, Smallwood DT. SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton. Molecular and Cellular Biology 2006;26(10):3738-51.