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

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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 components^{2,3}. 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 nanolevel^{5,12}. 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 re-

pair and regeneration²⁷⁻³¹. 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 cultures⁶⁵. Ascorbic acid, on the other hand, enriches the deposition of matrix with collagen⁶⁶, 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

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

Sources of MSCs	bone marrow, adipose tissue, synovial membrane, skeletal muscle, cord blood ¹⁹
Characterization of MSCs	Positively express : CD73, CD105, and CD90 Negatively express: CD34, CD45, CD14, CD11b, CD19, CD79 α , HLA- DR Osteogenic, Chondrogenic as well as Adipogenic differentiation capacity ²⁰
Multilineage differentiation capacity	osteocytes, chondrocytes, adipocytes ¹³ , skeletal muscle cells ²¹ , cardiac muscle ²² , smooth muscle cells ²³ , neural cells ²⁴ , hepatocytes ²⁵ , endothelial cells ²⁶

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

Osteoinductive Substances	References
Prostaglandin E2	Scutt et al. ³³ , Ninomiya et al. ⁴⁵
Sonic hedgehog	Spinella et al. ³⁴ , James et al. ⁴⁶ , Cai et al. ⁴⁷
Insulin- like growth factor- 1	Koach et al. ³⁵
BMP-2	Gori et al. ³⁶ , Jorgensen et al. ⁴³ , Piek et al. ⁴⁸ , Boehm et al. ⁴⁹ , Feng et al. ⁵⁰
BMP-6	Gruber et al. ³⁰ , Zhu et al. ⁴³
BMP-7	Gruber et al. ³⁸ , Qing et al. ⁵²
TGF-beta	Ng et al. ⁵³ , Zhou et al. ⁵⁴
FGF	Kiyono et al. ⁵⁵
Platelet Rich Plasma	van den Dolder et al. ⁵⁶ , Hu et al. ⁵⁷ , Elbackly et al. ⁵⁸
Dexamethasone	Wang et al. ⁵⁹ , Fiorentini et al. ⁶⁰
Vitamin D	Toai et al. ⁶¹ , Olivares-Navarrete et al. ⁶²
PTHr	Sammons et al. ⁶³ , Suriyachand et al. ⁶⁴

osteogenic effects via activation of Smad transcription factors⁶⁸, while parathyroid hormone induces the expression of osteogenic genes via the G-protein coupled receptor signaling pathway⁶⁹. 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

transplantations without any substantial risk for immune rejection⁷⁴. Evidence exists regarding the ability of MSCs to inhibit the proliferation of T-cells^{75,76} and B-cells⁷⁷. Moreover, MSCs can suppress the proliferation of natural killer cells, cytokine secretion and cytotoxic properties⁷⁸, in addition to inhibiting dendritic cell maturation and activation⁷⁹. An intermediate level of expression for MHC class I along with a very low level of expression in MHC class II (ref.⁸⁰) 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 niche⁸¹, 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” (ref.⁸²). This 3D system supports cell-to-cell interaction, cell migration and division, and cell differentiation via two types of signals: physical and chemical^{12,82}.

Chemical cues include growth factors, biomolecules, or any type of functional groups that bind to cell membrane receptors to support cell proliferation or differentiation^{12,83}. Physical cues, which are presumed to have a marked role in the differentiation of MSCs, are comprised of three subgroups: mechanical stimuli, electromagnetic, and topographical¹². Recent studies have demonstrated that mechanical properties of the extracellular environment trigger cell structure and function⁸⁴⁻⁹² and play a pivotal role in the regulation of tissue architecture and organization⁹³⁻⁹⁷. There are a large number of reports on osteogenic differentiation that can result from mechanical stimuli⁹⁸⁻¹⁰⁰, including compression^{84,101} and fluid shear stress¹⁰². Shear stress from the activation of mechanosensitive ion channels¹⁰³, Ca²⁺ channels^{104,105}, heterotrimeric G-proteins, and protein kinases¹⁰² enhances matrix mineralization and the expression of osteoblastic genes in human MSCs (ref.^{99,100,106,107}). 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^{108,109}.

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.

Fig. 1. Sequential events in commitment and osteogenic differentiation of mesenchymal stem cells.

Osteoinductive molecules (for example BMP2) stimulate the expression of RUNX-2, an early transcription factor in osteogenesis. Runx-2 commits MSCs to osteogenesis in one hand, and inhibits adipogenesis in the other hand⁷⁰. In the presence of this factor, MSCs express Dlx5, Msx2, P2Y₄, P2Y₁₄, and low level of ALP, Col I, and OPN (ref.^{71,72}). Then, these new committed osteoblast progenitor cells differentiate into spindle shape osteoblast cells in the presence of Runx-2 and β-catenin⁷³. These cells are capable to express some early bone specific genes, such as Bone sialoprotein, and OPN. At later stages, ALP and OPN support the maturation of osteoblasts. These fully differentiated osteoblast cells express bone specific markers such as Col I, ALP and OSC in high level and OPN in low level.

Abbreviations: BMP2, Bone Morphogenetic Protein 2; RUNX-2, Runt- related transcription factor- 2; MSCs, Mesenchymal Stem Cells; Dlx5, Distal-less homeobox5; Msx2, Mash homeobox homologue 2; P2Y receptors, Family of purinergic receptors stimulated by nucleotides; ALP, Alkaline Phosphatase; Col I, Collagen type I; OPN, Osteopontin; OSC, Osteocalcin

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 nanofibers¹¹⁰ and nanocrystalline hydroxyapatite (HAp) in the bone, native ECM is comprised of a complex mixture of pores and ridges of a nanometer scale diameter^{111,112}. 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 roughness¹¹³. This phenomenon consequently qualifies surface properties in the transmission of cell matrix signaling⁹⁴, cell activity^{12,114}, cell morphology¹¹⁵, adhesion¹¹⁶, motility¹¹⁷, and proliferation¹¹⁸, as well as gene regulation¹¹⁹. Based on Laurencin's study, cells attach better to fibers that have diameters smaller than their own¹²⁰. 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 regeneration⁸¹.

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^{5,121}. Research has shown that the surface properties of nanomaterials such as chemical characteristics, stiffness¹²²⁻¹²⁴ and nanotopography, in particular pits and grooves, has a tremendous impact on cell attachment and differentiation¹²⁵. 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. Nanoratings enhance adhesion but reduce the rate of proliferation of adhesive cells in comparison with planar substrates, while nanopits and nanoposts generally reduce cell attachment¹⁰². Nearly all types of cells, and MSCs in particular¹²⁶, align along the long axis of the grooves on substrates¹²⁶⁻¹²⁹. Although Clark et al. have shown that cell orientation increased with an increase in cell depth¹³⁰, Matsuzaka et al. have reported that rat MSCs followed the long axis in grooves >5µm width, yet prefer the bridge elongation in narrow grooves¹²⁹. These phenomena are organized by actin and other cytoskeletal elements¹³¹ and show that cells can recognize similarities in topography, especially at a nanoscale level¹³², thus replying in an appropriate manner. However, it is theoretically difficult to predict the effect of nanotopography on proliferation because there is a combination of different criteria, including geometry and substrate compositions that affect cell proliferation.

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 substrates¹³³. 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 osteopontin¹³⁴.

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-rod 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 samples¹³⁵. 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 polycaprolactone (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 chemicals¹³⁶. 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 differentiation¹³⁷.

Our group has also researched the effect of the nano-hydroxyapatite/poly (l-lactic acid) composite, with different morphologies on bone differentiation of MSCs (ref. ^{138,139}). Our results have shown that needle-like nano-hydroxyapatite/poly (l-lactic acid) composite provides the most appropriate matrix for producing bone constructs using MSCs (Fig. 2).

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 genes¹⁴⁰. Lock et al., also showed that Nano-

Fig. 2. Representative photomicrograph of rat marrow derived mesenchymal stem cells seeded in composite scaffolds.

A) Needle nHAp/ PLLA B) Spherical nHAp/ PLLA C) Rod nHAp/ PLLA (Bar= 50 μ m). Needle nHAp/ PLLA scaffolds provide the most appropriate matrix for proliferation of mesenchymal stem cells and producing bone constructs¹³⁸.

Abbreviations: nHAp, nano Hydroxyapatite ; PLLA, Poly- L- lactide

hydroxyapatite and nano-hydroxyapatite-PLGA composites provide a good alternative in directing the adhesion and differentiation of human MSC (ref.¹⁴¹). All these results provide evidence for the potential of nanobiomaterials, in particular for the promotion of osteogenesis.

The effect of surface nanobiomimetic properties of hydroxyapatite-coated genipin-chitosan conjugation scaffold on MSC cytoskeleton reorganization and osteogenic differentiation was evaluated by Wang and colleagues. They observed a significant difference in cytoskeletal organization and matrix mineralization between cells cultured into scaffolds with a nanostructure HAp surface and

those cells that were only cultured on the genipin-chitosan framework¹²⁵.

Not only ceramics, but also other nano-treated surfaces have been shown to have a direct effect on cell growth and osteogenic differentiation. The TiO₂-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 TiO₂ nano network¹⁴². 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, TiO₂-HAp coating surface property was able to induce osteogenic differentiation of human MSCs in the absence of chemical treatments¹⁴³.

Besides nanohydroxyapatite particles, nanofibers and nanotubes of different composition could enhance biomineralization when compared to solid-walled scaffolds¹⁴⁴. 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 system¹⁴⁵ 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 NANOTOPOGRAPHICAL 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 morphology¹⁴⁶ and the frequency of accessible sites for cell adhesion¹⁴⁷.

One of the key structures that manage the interaction between the cell and topography of the substrate are focal adhesions: FAs (ref.^{147,148}). Cells attach to their environments via FAs that are 15-30 nm in diameter¹⁴⁹. 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

Fig. 3. Cell response to mechanical cues via focal adhesion sites.

The assembly of focal adhesions in response to mechanical signals causes mechanical activation of mTORC2 which consequently phosphorylates and activates Akt. Activated Akt inhibits GSK3 β (ref.¹⁵²). Inhibition of GSK3 β leads to stabilization of β -catenin. The preserved β -catenin translocates into the nucleus¹⁵³ and acts as a transcription factor in expression of subsequent mechanoreponsive genes.

Abbreviations: mTORC2, Mammalian Target of Rapamycin Complex 2; PO₄³⁻, A Phosphate group; Akt (or Protein Kinase B), Ak (mouse strain) t (Thymoma); GSK3 β , Glycogen synthase kinase 3- beta.

behavior by showing that FAK deficient cells have lower cell stiffness, reduced adhesion strength, and increased cytoskeletal dynamics compared to wild-type cells¹⁵⁰. As signaling organelles, FA sites enable cells to touch their environment by transmitting mechanical and physical signals from the matrix to the cell^{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 factors¹⁵⁴. In order to probe their stability on the substrate, cells continuously apply small “cytoskeletal traction forces” on FA sites¹⁵⁵. If opposing external force (from the ma-

trix) and internal physical forces (from the cell) are unequal, cell surface adhesion clusters will start moving to achieve to a stable position¹⁵⁶. There are a large number of studies showing that nanotopographical cues modulate integrin clustering and the formation of FAs (ref.¹⁵⁷⁻¹⁵⁹). Any changes in their density are linked to changes in stem cell differentiation¹⁶⁰⁻¹⁶². 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 cues¹⁶³. 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 adhesions¹⁵⁸. Nanotopographic features may lead to changes in the number and arrangements of FAs (ref.¹⁶⁴). This asymmetric distribution then transmits signals to the cell via connections between FAs and cyto-nucleoskeletal proteins¹¹⁴. In other words, these cell-matrix adhesion sites (FAs) transduce mechanical stress into chemical signals inside the cells¹⁶⁵. There is a correlation between the number of FAs and the density of matrix proteins that contain the RGD motif¹⁶⁶. The beta-integrin subunit of FA is associated with FAK, which is a non-receptor tyrosine kinase¹⁶⁷. This enzyme influences the cell transcriptome profile and regulates stem cell differentiation¹⁶⁸ via the adhesion-dependent phosphorylation of downstream protein kinases such as extracellular signal regulated kinase: ERK (ref.^{169,170}). It seems that transferring ERK 1/2 from cytoplasm to the nucleus is the principle event in the modulation of differentiation¹⁵⁷, control of cell proliferation^{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.¹⁷³⁻¹⁷⁷). Shih et al. showed that activities of FAK and ERK1/2 kinases increase on stiffer substrates during osteogenic differentiation¹⁷⁸. 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”. Any changes in expression of Zyxin, eventually modulates gene expression and cytoskeletal reorganization¹⁷⁹.

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.^{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^{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 progression¹⁸⁰⁻¹⁸⁶, 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 contraction¹⁸⁷. Changes in actin dynamics are monitored by myocardin-related tran-

Fig. 4. Relationships between mesenchymal stem cell adhesion molecules and the surface of nanomaterials.

Discontinuity in the topography of biomaterials leads to changes in cell morphology and the frequency of accessible sites for focal adhesions. Externally applied mechanical stress is linked to actin networks via these focal adhesions. Any changes in the distribution of focal adhesions and actin networks are translocated to the nucleoskeleton and consequently affects on the expression of corresponding mechano-responsive genes.

Abbreviations: FAs, Focal Adhesions; G-Actin, Globular Actin; F-Actin, Filamentous Actin; LIMK, (Lin11/Isl1/Mec3) Kinase; P120 or Catenin Delta 1, A prototypic member of Armadillo protein family; ROCK, Rho-associated protein kinase; Src (Sarcoma), A proto-oncogene which encodes non- receptor tyrosine kinases

scription factor A (MRTF). This type of protein, which is found in both cytoplasm and nucleus, interacts with G-actins¹⁸⁸⁻¹⁹⁴. In the nucleus the inactive form of MRTF is bound to G-actin. It is assumed that cytoplasmic and nuclear G-actins are sensed by shuttling MRTF. In other words,, this transcriptional regulator is the sensor which links externally applied mechanical stress to the actin network, and consequently to the nucleus, so this transcriptional regulator, indirectly affects on the expression of corresponding mechano-responsive genes in the nucleus (Fig. 4).

On the other hand, ERM proteins (Ezrin, Radixin, and Meosin) are known to have roles in modulation of human MSCs biomechanics. Activated ERM proteins bind to F- actin filaments and cell adhesion molecules. Silencing the expression of ERM genes causes disassembly of actin fibers and FAs. These mechanical changes subsequently lead to impaired osteogenic differentiation¹⁹¹.

It is also assumed that any changes in the distribution of FAs and cytoskeleton remodeling are translocated to the nucleoskeleton via bridging proteins, including SUN and nesprins, and hence to the DNA through matrix attachment regions¹⁹². It can thus be inferred that any physical cue on the outer surface of the cell can be transferred to the nucleus in order to exert its effect on chromosome positioning, and thus effects on positioning of transcription factors on DNA and the expression of specific genes.

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; Ca⁺² 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-3 β , Glycogen synthase kinase 3- beta; GTP, guanosine triphosphate; HAp, hydroxyapatite; LIMK, (Lin11/Isl1/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; PO₄³⁻, 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 MAD (mothers against decapentaplegic); Src (Sarcoma), A proto- oncogene which encodes non- receptor tyrosine kinases; SUN proteins, Sad1p, UNC-84; TCP, Tricalcium Phosphate; TGF- β , Transforming Growth Factor- β ; 3D, Three Dimensional; TiO₂, Titanium Oxide.

CONFLICT OF INTEREST STATEMENT

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

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