World Journal of Stem Cells

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DOI: 10.4252/wjsc.v7.i2.266

World J Stem Cells 2015 March 26; 7(2): 266-280
ISSN 1948-0210 (online)

REVIEW

Role of nanotopography in the development of tissue engineered 3D organs and tissues using mesenchymal stem cells

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Author contributions: All authors contributed equally to the manuscript in terms of writing the paper.
Conflict-of-interest: Authors confirm that no conflict of interest exists regarding this manuscript.

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Received: July 29, 2014
Peer-review started: July 30, 2014
First decision: September 29, 2014
Revised: November 7, 2014
Accepted: December 3, 2014
Article in press: December 10, 2014
Published online: March 26, 2015

Abstract

Recent regenerative medicine and tissue engineering strategies (using cells, scaffolds, medical devices and gene therapy) have led to fascinating progress of translation of basic research towards clinical applications. In the past decade, great deal of research has focused on developing various three dimensional (3D) organs, such as bone, skin, liver, kidney and ear, using such strategies in order to replace or regenerate damaged organs for the purpose of maintaining or restoring organs' functions that may have been lost due to aging, accident or disease. The surface properties of a material or a device are key aspects in determining the success of the implant in biomedicine, as the majority of biological reactions in human body occur on surfaces or interfaces. Furthermore, it has been established in the literature that cell adhesion and proliferation are, to a great extent, influenced by the micro- and nano-surface characteristics of biomaterials and devices. In addition, it has been shown that the functions of stem cells, mesenchymal stem cells in particular, could be regulated through physical interaction with specific nanotopographical cues. Therefore, guided stem cell proliferation, differentiation and function are of great importance in the regeneration of 3D tissues and organs using tissue engineering strategies. This review will provide an update on the impact of nanotopography on mesenchymal stem cells for the purpose of developing laboratory-based 3D organs and tissues, as well as the most recent research and case studies on this topic.

Key words: Nanotopography; Mesenchymal stem cells; Tissue engineering; Nanotechnology; Three dimensional organs/tissues; Scaffolds

Core tip: Tissue engineering and nanotechnology are both exciting fields that have enormous potentials to revolutionise medicine as we know it today. Use of nanotechnology is an attractive and effective way to control and direct biological events at cellular levels. Nanoscale architecture plays a pivotal role directing cellular activities. Here, the use of nanotopography for the purpose of 3D organ/tissue regeneration using mesenchymal stem cells (*i.e.*, their proliferation, differentiation and function), is reviewed by investigating the most recent, innovative, and effective studies in this field.

Salmasi S, Kalaskar DM, Yoon WW, Blunn GW, Seifalian AM. Role of nanotopography in the development of tissue engineered 3D organs and tissues using mesenchymal stem cells. *World J Stem Cells* 2015; 7(2): 266-280 Available from: URL: http://www.wjgnet.com/1948-0210/full/v7/i2/266.htm DOI: http://dx.doi.org/10.4252/wjsc.v7.i2.266

INTRODUCTION

It is becoming progressively evident that with an ever increasingly older population and high costs associated with meeting the healthcare demands[1] as well as the shortage of organs and effective therapeutic methods[2], the field of medicine has to move towards cutting edge, laboratory engineered techniques and devices if, we are to avoid a drastic world-wide healthcare collapse in the near future. To this end, the fields of nanotechnology, regenerative medicine and tissue engineering (TE) are expanding at a rapid pace. Recent regenerative medicine and TE strategies (using cells, scaffolds, medical devices and gene therapy) have led to fascinating progress of translation of basic research towards clinical applications[1,3]. In the past decade, great deal of research has focused on developing various three dimensional (3D) organs, such as bone[4], skin[5], liver[6], kidney[7], and ear[8], using such strategies in order to replace or regenerate damaged organs for the purpose of maintaining or restoring organs' functions.

Human organs are responsible for various important functions of the body including, but not limited to, digesting food, serving as a barrier against infections, recognising and coordinating the body's response to its internal and external environmental changes, and providing oxygen (to be used for cellular respiration) as well as removing excess carbon dioxide. They are also responsible for maintaining homeostasis, transmission of information and generating force[3]. In cases when one or a few of the organs are severely damaged, to an extent that they are no longer capable of reconstructing or regenerating themselves, tissue engineering and nanotechnology based strategies, using previously established knowledge on cellular behaviour[9,10], could be employed to develop and construct tailored therapies, devices, or even whole organs. To this end, nanostructured, bio-inspired, or biological materials have attracted a great deal of attention as they poses unique chemical, mechanical and surface characteristics that could prove useful for organ or tissue TE.

The surface properties of a material or a device are key aspects in determining the success of the implant in biomedicine, as the majority of biological reactions in human body occur on surfaces or interfaces[11]. Furthermore, it has been established in the literature that cell adhesion and proliferation are, to a great extent, influenced by the micro- and nano-surface characteristics of biomaterials and devices[12,13]. In addition, it has been shown that the stem cells', mesenchymal stem cells (MSCs) in particular, functions can be regulated through physical interaction with specific nanotopographical cues[3,14], further indicating the importance of surface characteristics at nanometre length scale. Therefore, guided stem cell proliferation, differentiation and function are of great importance in the regeneration of 3D organs and tissues using TE strategies.

This review will provide an update on the impact of nanotopography on MSCs for the purpose of developing laboratory-based 3D organs, as well as the most recent research and case studies on this topic.

NANOFABRICATION OF 3D SCAFFOLDS WITH STEM CELLS

Previously, most research and investigations focused on growing cells in a petri dish (2D). However, in nature cells use 3D template of extracellular matrix (ECM) to shape functional tissues[15]. Micro- and nano-scale chemical and physical cues from the ECM environment control and direct various key cell behaviours including their adhesion, proliferation, migration and differentiation[16-18]. Therefore, the construction of a synthetic system that mimics the natural ECM and its component has become a field of topical interest[19].

Recent investigations have shown rapid success in TE of sophisticated and complex nanoenvironments suitable for 3D growth of stem cells for the purpose of organ and tissue regeneration[19-22]. So far, various biofabrication techniques have been developed and employed to design an ideal 3D synthetic ECM-mimetic system that resembles the architecture and mechanical properties of the natural ECM[3]. Natural or synthetic polymers are used as scaffold materials and, depending on their nature, suitable biofabrication techniques are used to create a 3D environment with nanotopographical cues that can lead to controlled and directed growth and differentiation of stem cells toward a specific tissue or organ regeneration. Numerous studies have covered the currently available fabrication techniques for natural or synthetic polymers[15,23-27]. In general, the available fabrication techniques can

Table 1 Classification of various types of nanotopography (nanofabrication) methods

Energy source	Method	Mechanism and final outcome	Processable polymers
Thermal	Replica modelling	Creating negative shape of the mold by thermal cross-linking of cavity-filled pre-polymer	Thermocurable polymers, *e.g.*, poly(dimethyl siloxane)
	Nanoimprint lithography	Creating negative shape of the mold by plastic deformation of polymer above Tg	Thermoplastic, *e.g.*, polystyrene, poly(lactic acid), and conductive polymers, *e.g.*, polyaniline and polypyrrole
	Block copolymer lithography	Creating nanoscale hole, line and lamellar structures by microphase separation of two immiscible polymers	Block copolymer, *e.g.*, polystyrene-block-poly(methyl methacrylate), styrenebutadiene-styrene
Optical	Photolithography	Depending on mask design and selective UV exposure, solubility is changed	Photo curable polymers, *e.g.*, photoresist, polyurethane-based
	E-beam lithography	Formation of arbitrary patterns using different electron beam pathways and selective irradiation of focused electron beams to change solubility	E-beam sensitive polymers, *e.g.*, polymethyl methacrylate
	Direct laser writing	Formation of arbitrary patterns by selective cross-linking of the polymer by laser irradiation	Photo-curable polymers
Chemical	Microcontact printing	Creating extruded patterns of elastomeric stamp using relative surface energy difference needed for transferring materials	Proteins and self-assembled monolayers
	Dip-pen lithography	Formation of arbitrary patterns by direct writing of molecules with a sharp tip	Self-assembled monolayers
	Salt leaching/gas foaming	Formation of a block of polymer with voids by dissolution of salt particles (salt leaching) and/or bubble formation in the polymer block (gas foaming)	Solvent soluble polymers, *e.g.*, thermoplastic and conductive ones
Electrical	Electrochemical deposition	Forming negatively shaped molds by electrochemical reduction of the polymer	Conductive polymers
	Electrospinning	Drawing a three dimensional nanofibrous mesh from the polymer solution using an electric field	Solvent soluble polymers
Physical	Capillary force lithography	Formation of partially filed negative shape of the mold by capillary rise of thermoplastic polymer above Tg	Thermoplastic and solvent soluble polymers
	Micromolding in capillaries	Creating a negative shape of the mold by capillary-driven microchannel filling	Solvent soluble polymers
	Wrinkle	Formation of random or aligned micro- or nanolines using mechanical buckling Mechanical buckling between elastic substrate and rigid film	Elastomeric polymers, *e.g.*, polydimethylsiloxane
	Crack	Formation of aligned or inter-crossing line patterns by mechanical fracturing of the stiff film adhered onto elastic substrate	Elastomeric polymers

Adapted from Kim *et al*[3].

be classified into different categories based on their energy source, *i.e.*, thermal, optical, physical, chemical or electrical (Table 1). It is beyond the scope of this paper to review the different available techniques in each of these categories, however, a review conducted by Kim *et al*[3] could be referred to for further and in detail information on this topic.

CHARACTERISTICS OF MSCs AND THEIR APPLICATION IN TE OF 3D ORGANS

An extensive number of studies have demonstrated the great potentials of using MSCs for TE approaches[28-33]. Among many advantageous characteristics of MSCs the fact that these cells can be isolated from several tissues and that they have the potential to be expanded in culture and exhibit multilineage differentiation (Figure 1) make MSCs a highly interesting stem cell source for TE and regenerative medicine research[31]. Other interesting properties of MSCs include; their ability to self-renew, modulate immune responses, and their availability (they can be obtained from a small scale aspirate of bone marrow or adipose tissues)[34-36]. Furthermore, MSCs can be isolated from adults, therefore, allogeneic transplant of these cells would eliminate raising ethical issues in regards to their use in TE and regenerative medicine[36].

Among most potential characteristics of MSCs, it is probably their ability of multilineage differentiation that is mostly exploited for TE and regenerative medicine purposes. The differentiation of MSCs is controlled by some regulatory genes and induction chemicals that lead to the specific differentiation of these progenitor cells[37,38]. In addition to growth factors and induction chemicals, various biomaterials (*i.e.*, natural and synthetic polymers) are used to provide appropriate scaffolding for the proliferation and differentiation of MSCs for the purpose of reconstruction of several hard and soft tissues and organs, such as bone, cartilage, tendons, and skin[39,40] (Figure 2).

THE ROLE OF NANOTOPOGRAPHY ON THE GROWTH AND PROLIFIRATION OF MSCS

As mentioned earlier, surface nanotopography of

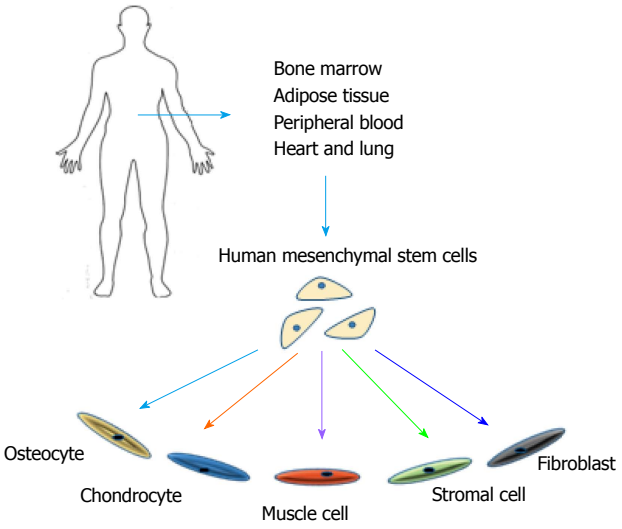

Figure 1 Potentials and sources of mesenchymal stem cells. Mesenchymal stem cells can be collected from various sources within human body and have the ability to differentiate into a variety of lineages.

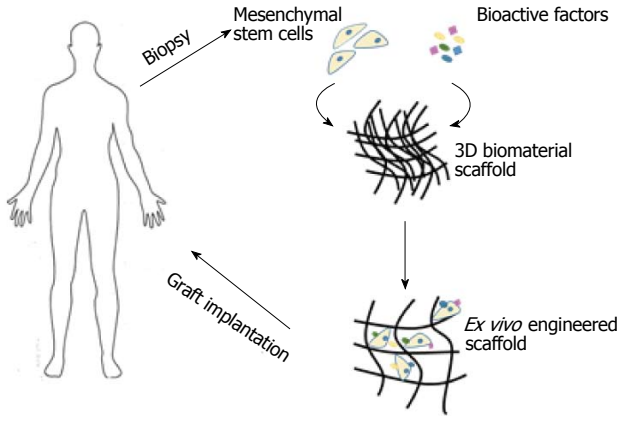

Figure 2 Overview of tissue engineering strategy of incorporating scaffolds with mesenchymal stem cells. Mesenchymal stem cells (derived directly from the patient) are expanded in the laboratory, whereby the necessary environment for their growth has been prepared. These cells are then seeded onto a scaffold and either allowed to differentiate *ex vivo* pre-implantation or the scaffold is immediately implanted.

biomaterials can evoke specific cellular responses. Materials with unique nanotopographical characteristics offer properties, similar to growth factors, which can be used to induce specific biological performances of safe and cost effective manners in the human body[41]. Previous studies show that various nanotopographical cues can potentially impact the adhesion[42,43], orientation[44], and cytoskeletal organisation[45] of MSCs as well as their self-renewal[46], proliferation and differentiation[41]. Furthermore, nanotopographical cues could influence morphology, migratory capacity, gene expression and subsequently the fate of MSCs[47,48].

It has been shown that nanofeatures including nanopits, nanogratings and nanoprotrusions have the potentials to influence the cell morphology, proliferation and differentiation of MSCs[49,50] (Figure 3). For instants, it has been shown that homogenously nanopatterned and chemically modified surfaces can have direct effect on cellular responses of MSCs, including their self-renewal abilities, control over their initial cell interactions and subsequently their cell phenotype, by creating arrays of nanodots using dip pen nanolithography[51] (Figure 4). Furthermore, differentiation and proliferation of human MSCs (hMSCs) were investigated on nanogratings of 350 nm width combined with biochemical cues such as retinoic acid, and it was shown that synthetic nanostructures can induce hMSCs to differentiate into neuronal lineage[52]. This study, conducted by Yim *et al*[52], also confirmed the significance of nanotopography as it revealed that retinoic acid alone on unpatterned surfaces did not lead to strong neuronal marker expression as it was shown on surfaces with nanogratings. Other nanopatterned structures, such as grooves, ridges, and pores as well as holes, nods, or rods are of other commonly techniques currently employed to change unpatterned surfaces for MSCs to grow on and to direct their cellular responses[49]. Such nanostructures have great applications to all areas of TE. For instance, Andersen *et al*[53] investigated adhering nanoparticles containing different small-interfering RNAs (siRNAs) into nanostructured scaffolds consisting of nanopores and reported of spatial retention of the RNAs within nanopores seeded with MSCs, which resulted in enhanced osteogenic and adipogenic differentiation of MSCs[53]. This is an exciting finding as the ability of directing a single type of differentiation plays a crucial role in developing specific 3D organs. In another study, Watari *et al*[54] used topographically-patterned substrates containing anisotropically ordered ridges and grooves to modulate osteogenic differentiation in hMSCs[54]. They reported that hMSCs cultured on 1400 or 4000 nm pitches, compared to those seeded on 400 nm pitch or planer control, exhibit better elongation

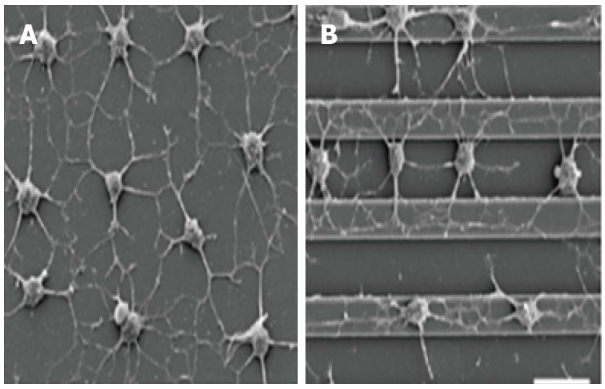

Figure 3 Comparison of different topography strategies employed to investigate the effects of anisotropic *vs* isotropic cytoskeletal tension on cultured mesenchymal stem cells. (A) nonpatterned substrates caused randomly oriented cell protrusions to be formed, while (B) alignment of elaborated processes in the direction of the grooves were induced by micropatterned surfaces, mimicking the native structure and orientation of the natural extracellular matrix proteins[52].

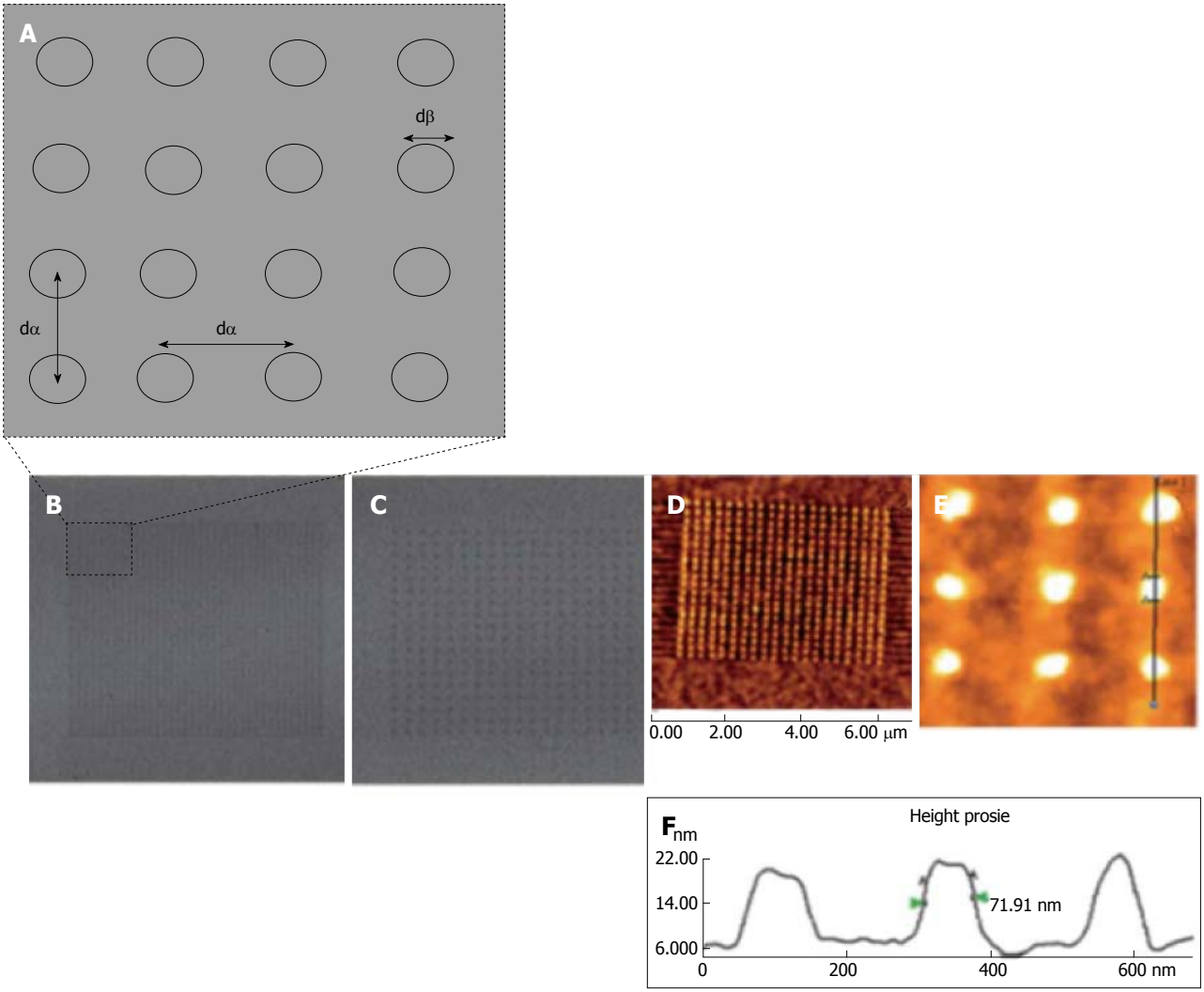

Figure 4 Nanopatterned gold surfaces examination for the effect of both the nanotopography and terminating chemical functionality. A: Nanopatterned surfaces used for mesenchymal stem cell control and differentiation exhibiting dot to dot pitch (dα) and dot diameter (dβ); B: Lateral Force Microscopy (LFM) image of small area 280 nm pitch array; C: LFM image of 140 nm pitch array; D-F: Atomic force microscopy topographical image of an alkanethiol resist array fabricated on gold surface following chemical etching. An average diameter feature (dβ) of 70 nm was shown on the cursor profile[49].

and alignment, while they showed a significant decrease in Runt-related transcription factor 2 (RUNX2) and bone gamma-carboxyglutamic acid-containing protein (BGLAP) expression. Their data also revealed that 400 nm pitch increased extracellular calcium deposition. Watari *et al*[54] concluded that specific size scale of topographic cues could directly influence the osteogenic differentiation of hMSCs both with and without osteogenic agents. This is another important finding that could enable one to manipulate and develop nanostructures that could lead to controlled and directed differentiation of stem cells for the purpose of TE of 3D organs. Very recently, the effect of topographical design, in the form of nano-pillar, nano-hole and nano-grill, on hMSCs were investigated by Wu *et al*[55] in which these nanotopographies were applied onto a polycaprolactone surface using thermal nanoimprinting. Their findings revealed that nanotopographical patterns trigger changes in the morphology and cytoskeletal structure of hMSCs. They also found that, compared to non-patterned surfaces, nano-pillar and nano-hole topography determined MSCs chondrogenesis, resulting in specific cartilage formation. Furthermore, Kilian *et al*[56] showed that geometric nanotopography cues, that increase actomyosin contractility, could influence and direct the osteogenesis of bone marrow-derived hMSCs. Such geometric cues direct and control mechanochemical signals and paracrine/autocrine factors necessary for specific differentiation of MSCs, also observed during the *in vivo* investigation of the microenvironment of the differentiated cells.

CASE STUDIES ON THE APPLICATION OF NANOTOPOGRAPHY GUIDED TE OF 3D ORGANS/TISSUES USING MSCS

Bone

Reconstruction of large bone defects caused by

surgery, trauma or tumours are common deficiencies, which present a significant medical challenge[57]. Autologous bone grafting is the gold standard for treating bone defects, but obstacles such as limited sources of tissue, and bone resorption before bone healing, have raised interests in synthetic materials as potential bone substitutes[58]. Furthermore, bone grafting has proven challenging for large bone defects reconstruction[59]. This is mainly due to difficulties in harvesting enough bone grafts from a healthy bone, potential postoperative pain, risk of infection, risk of hypersensitivity, risk of paresthesia, and time constraints[60,61]. TE, using stem cells, provides the opportunity to avoid the established drawbacks of bone graft materials for the purpose of reconstructing or regenerating bone defects at variety of scales. MSCs, because of their interesting properties, have been demonstrated as an attractive cell source for bone TE applications[62]. Controlled and directed differentiation of MSCs into osteoblasts (bone cells) is therefore a key aspect of this process. As mentioned above, nanotopographical cues could be used to influence MSCs cell behaviour and differentiation toward specific lineages. For instance, in a very recent study, McCafferty *et al*[63] demonstrated the use of nanotopography to induce osteogenic differentiation of human bone marrow derived MSCs. They sputter deposited thin films of bioactive calcium phosphate onto a polycrystalline titanium nanostructured surface. These sputter deposited surfaces supported high levels of bone marrow–derived hMSCs proliferation and adhesion, determined by DNA quantification. Moreover, they were also able to directly promote significant levels of osteogenic differentiation. In this study, gene expression, alkaline phosphatase activity and immunocytochemical localisation of key osteogenic markers showed that the nanostructured titanium surfaces and the bioactive calcium phosphate coatings could direct differentiation towards an osteogenic lineage. The addition of the calcium phosphate chemistry to the topographical profile of the titanium was found to induce increased bone marrow–derived hMSCs differentiation compared to that observed for either the titanium or calcium phosphate coating without an underlying nanostructure. Therefore, the results presented a clear benefit from a surface engineering strategy that combines a defined nanoscale surface topography with a conformal bioactive chemistry. In another study by de Peppo *et al*[64] osteogenic response of hMSCs to titanium-coated hemisphere-like topographic nanostructures of 50, 100, and 200 nm was assessed. Their aim was to look at the influence of different sizes of nanoscale topographies on the morphology, proliferation, and osteogenic differentiation of hMSCs *in vitro*. Here, the nanostructuring was fabricated using colloidal lithography and the desired structure sizes were achieved by etching the original 200 nm polymeric particles (polystyrene particles) and further heat-treating them above the transition temperature of polystyrene (118 ℃), to create semispheroidal nanoparticles to increase their surface attachment abilities. Their results showed that there is a direct relationship between the proliferation and osteogenic differentiation of hMSCs and the size of the underlying structures, demonstrating that by varying the scale of the nanotopographic features at nanoscale, one can control the osteogenic behaviour of hMSCs. de Peppo *et al*[64] concluded that colloidal lithography in combination with coating technologies can enable structuring well defined nanoscale topographies to control and direct hMSCs growth and differentiation. Dalby *et al*[65] also investigated the osteogenic response of hMSCs to semi-ordered and random nanotopographies performed by colloidal lithography and polymer demixing on silicon and showed that hMSCs react robustly to nanotopographic features down to 10 nm in size with a low aspect ratio[65]. In this study, scanning electron microscopy of primary hMSCs on flat controls and scaffolds with nanotopographic structures showed that hMSCs exhibited strong reaction to nanofeatures as their filopodia extended and curled around these features (Figure 5). Dalby *et al*[65] concluded that their recorded osteogenic response of hMSCs to nanotopographies could be employed to construct and design scaffolds with an appropriate osteogenic "environment" instead of planar control structures in order to direct and control MSCs growth and differentiation.

Furthermore, Rosa *et al*[66] examined the osteoinductive potential of titanium (Ti) surfaces with nanotopographic features, yield by chemically treating polished Ti discs with H_2SO_4/H_2O_2, and cultured them with rat MSCs under osteogenic and non-osteogenic conditions. Untreated polished Ti surfaces were used as controls. Their findings revealed that Ti surfaces with nanotopography boosted cell proliferation and alkaline phosphatase (Alp) activity of rat MSCs under both osteogenic and non-osteogenic conditions (Figure 6). They also demonstrated that nanotopography upregulated the gene expression of major bone markers under both of the test conditions. Interestingly, they noticed that obtustatin, an $\alpha 1\beta 1$ integrin inhibitor, was able to reduce higher gene expression of key bone markers and Alkaline Phosphatase (ALP) activity on Ti Scaffolds with nanotopographic features. Therefore, suggesting that obtustatin signalling pathway plays a crucial role in determining the osteoinductive effect of nanotopography on MSCs, a finding that can be exploited as a novel mechanism of accelerating and/or enhancing MSCs osseointegration for the purpose of TE of complex organs or tissues in the future.

Cartilage

Cartilage defects, caused by osteoarthritis, trauma or sport, are considered serious clinical problems. So far,

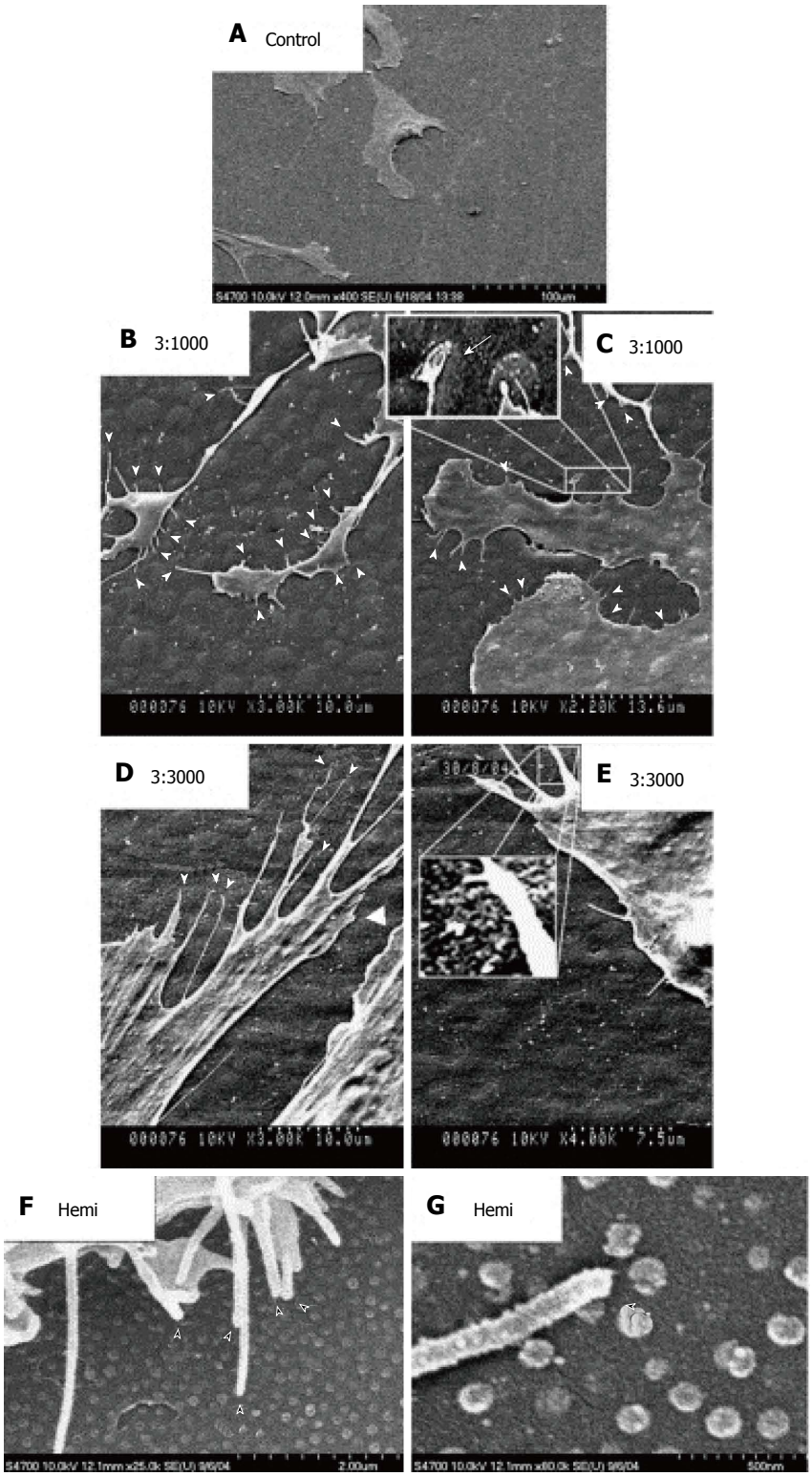

Figure 5 Scanning electron micrographs of human mesenchymal stem cells cultured on control and test materials. A: On planar control materials cells showed normal morphologies; B, C: Filopodial of the human mesenchymal stem cells (hMSCs) interacts with the 3:1000 substrates (arrowheads); C: hMSC filopodia are curving around an island; D, E: Filopodial interactions with the 3:3000 substrates (arrowheads); E: A filopodia curving around an island is clearly observed; F: filopodial interactions with the hemi substrates (arrowheads); G: Filopodia curving around a hemisphere (arrowhead)[65].

TE of cartilage has proven to be much more difficult than many other organs or tissues, due to cartilage's inherently poor regenerative ability[67]. Therefore, developing a functional TE system, capable of improving the regenerative ability of this tissue, would be of great interest. Most research on this field have been focused on using polymeric scaffolds with stem cells, in particular MSCs[8,67-69]. MSCs are considered the "gold standard" of stem cell source for cartilage TE as their differentiation to chondrocytes can be easily controlled and directed using various techniques, in particular nanotopography[8,55].

Previous studies have shown that a more rounded, spheroidal cell shape can enhance the rate of chondrogenesis, through increasing the expression of chondrocyte-related genes, markers, and proteins[70,71]. Based on this, Zhong *et al*[72] attempted to create a microenvironment suitable for MSCs fibrochondrogenesis

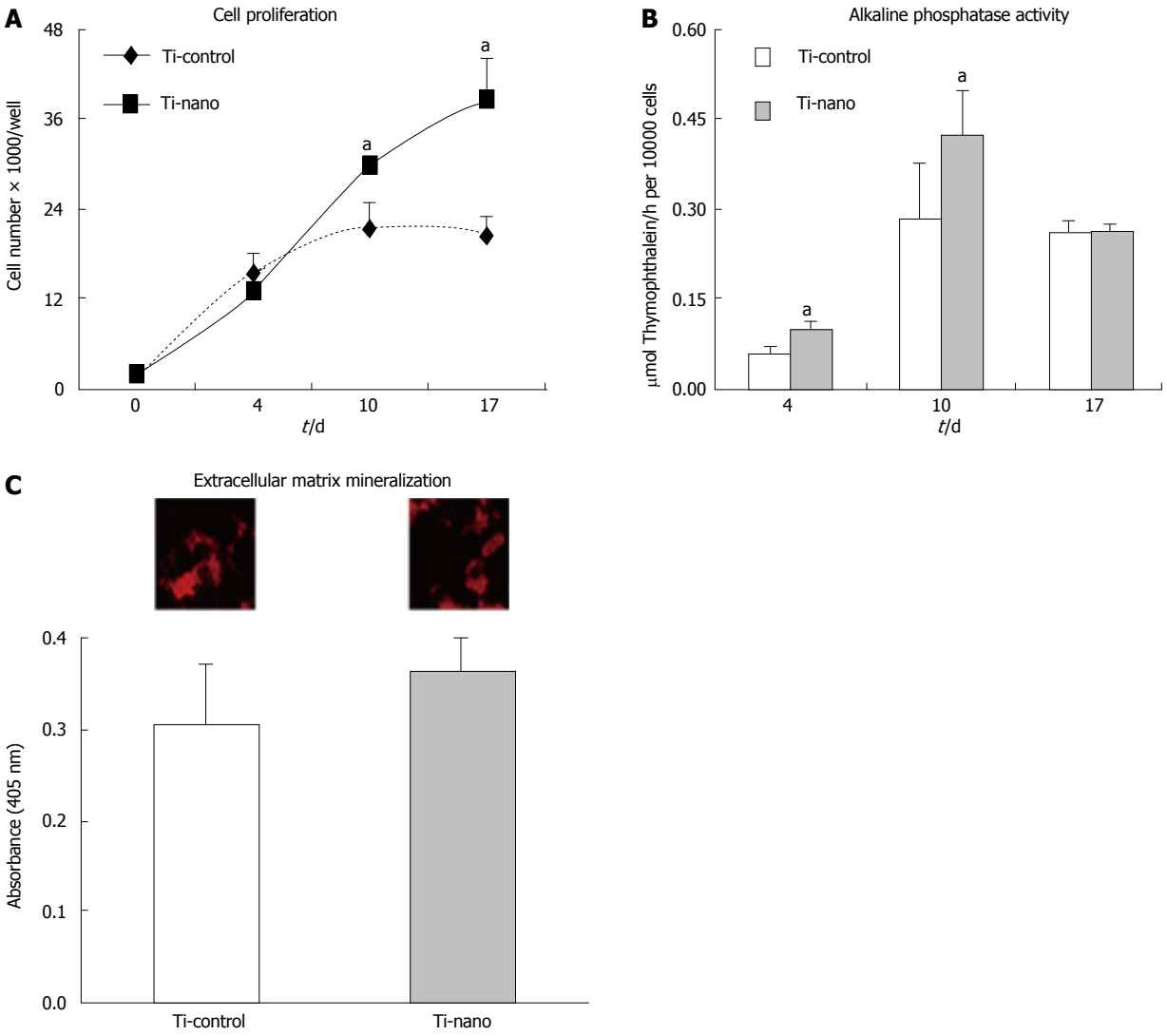

Figure 6 Investigation of the effects of nanotopography on proliferation. (A), alkaline phosphatase (Alp) activity (B), and extracellular matrix mineralization (C) of mesenchymal stem cells differentiated into osteoblasts and cultured on nanotopography in an osteogenic medium compared to control Ti surfaces. A: The number of cells was significantly increased on Ti with nanotopography on days 10 (P = 0.07) and 17 (P = 0.03); B: Higher Alp activity was supported by Ti surface with nanotopography supported on days 4 (P = 0.01) and 10 (P = 0.04); C: The difference in the level of calcium mineralisation in the matrix (insets) was not statistically significant (P = 0.13) by comparing both surfaces. The data are presented as mean ± standard deviation (n = 4). [a]Indicates statistically significant difference[66].

using simultaneously integrated nanotopography and flow stimulus. They developed a biomimetic microfluidic device consisting of aligned nanofibers of poly lactic-co-glycolic acid (PLGA), and micorchambers of different angles. The micorchambers were used to enable flow direction to create different angles with PLGA nanofibers. Their findings showed that the combination of fluid flow, nanotopography-induced cues, and the direction of flow in relation to the aligned nanofibers contributed towards the round shape morphology of MSCs, associated with fibrochondrogenesis during chondrogenic differentiation of these cells.

Although, most studies have shown positive contributions of various nanotopographical cues on MSCs growth and differentiation, a few studies have concluded that certain types of nanotopography could have adverse effect on the differentiation of MSCs into chondrocytes for the purpose of repairing or regenerating cartilage. The study by Wu *et al*[55] is a good example on this statement as their findings revealed that MSCs experienced delayed chondrogenesis on samples with nano-grill topography. They observed radically different morphological, proliferation and chondrogenesis changes as well as significantly higher upregulation of type Ⅱ collagen on nano-pillar and nano-hole surfaces compared to nano-grill surfaces, where the expression of collagen Ⅰ marker was drastically higher (Figure 7). Similarly, Trujillo *et al*[73] reported of decreased chondrogenic differentiation of adipose-derived MSCs on nanowire surfaces. They used sintering and solvent-free nanotemplating to fabricate polycaprolactone (PCL, as control) and nanowire (NW) samples. After 4 and 7 d of culturing the samples with adipose-derived MSCs, both groups demonstrated positive support for cell attachment and proliferation. Once chondrogenic differentiation

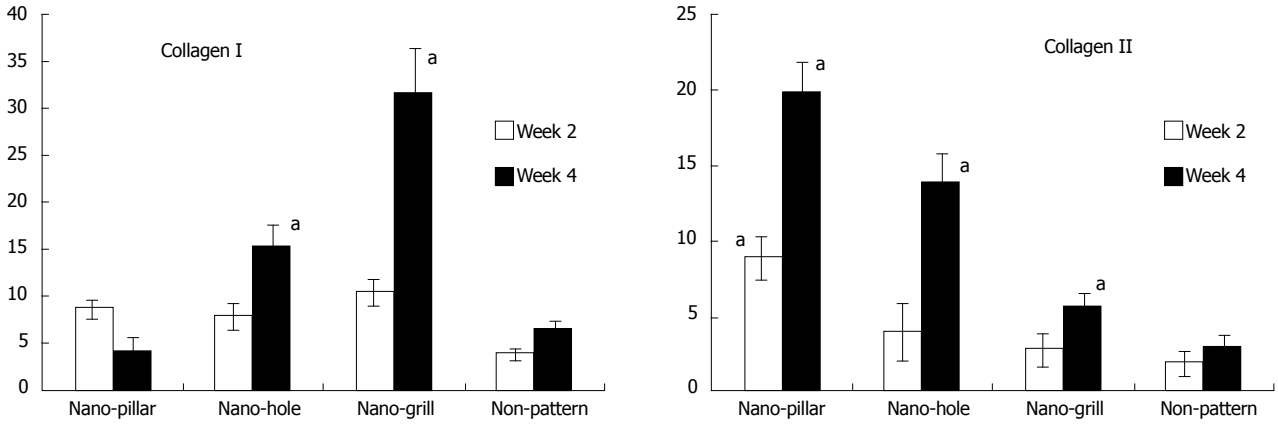

Figure 7 The effect of nano-patterned surfaces on the expression of cartilaginous genes. Real time polymerase chain reaction was used to analyse mRNA expression levels of cartilaginous genes at week 2, 4 or 6 of chondrogenic differentiation, which was normalised to their respective glyceraldehyde-3-phosphate dehydrogenase expression and expressed as fold changes relative to undifferentiated mesenchymal stem cells. n = 3 per group, mean ± SD. $^{a}P < 0.05$ which was considered statistically significant compared to non-patterned surface[55].

media was supplemented to the cultures, alcian blue staining was used to confirm the presence of acidic polysaccharides, such as sulphated glycosaminoglycans, normally found in articular and hyaline cartilage tissue. At 3 wk, it was evident that there had been significantly higher production of polysaccharides on PCL compared to NW. The authors also investigated PCL and NW samples under adipogenic differentiation conditions and found the results to be reversed, *i.e.,* there was increased adipogenic differentiation of adipose-derived MSCs on the NW samples. These findings indicate that nanotopography can have bias and in some cases unexpected effects on the differentiation of MSCs towards a particular lineage. However, further research is required on this particular topic in the future, in order to better understand the underlying mechanism of such adverse events.

Skin

Millions of burn injuries occur worldwide that cause serious harm to skin and subsequently to the general health of patients, as the first line of a patient's defence is compromised. In cases where the injuries are too severe for the natural repair process to take place, skin TE is considered. Skin, with a surface area of 1.8 m^2, is the largest organ in the body, which consists of two layers: the outer protective epidermis and inner corium or dermis. Currently the main obstacle in front of skin TE is *in vitro* culture time required to grow epithelial sheets large enough to be used for severe cases. This is particularly dangerous as the longer the wound takes to heal, the patient is at higher risks of acquiring infection[74]. Another issue is that epithelial cells are very sensitive and adhering them to the burned surfaces is very difficult[75].

Like in other diseases, stem cells can help improve the healing and regenerating process of skin. Previously, bone marrow-derived MSCs have been shown to differentiate into epithelial cells of skin[76], and promising results have been achieved in treating skin wounds, especially chronic ones[77,78]. Wounds treated using bone marrow-derived MSCs have shown accelerated wound closure with rapid re-epithelisation, cellularity and angiogenesis[79]. Incorporation of these cells into a suitable scaffold, which closely mimics the native micro- and nanotopographical characteristics of the ECM of the skin, may offer improved opportunity to repair or regenerate skin[80]. For this to happen, two events should take place; (1) MSCs should be directed to the sites of injury; and (2) they should differentiate into cells of skin lineage. This is achievable by designing scaffolds with specific nanotopographical features[81].

Based on the highly oriented nanogrooved structures of natural ECMs in human body, Kim *et al*[82] designed nanotopographically variable grooved matrices, using UV assisted capillary force lithography, with curable polyurethane acrylate (PUA) polymer. The PUA nanogrooved matrices were then gelatine coated prior to cell culturing with hMSCs. In this study, the effect of nanotopographical density was investigated on hMSCs migration and proliferation for wound healing purposes. It was shown that hMSCs migrate into the target area (the wounded, cell free area) and that the hMSCs on nanogrooved matrices exhibited a significantly higher speed of cell migration than those on the flat controls. They also investigated various densities of nanogrooves and found that as the density of the nanogrooved matrices increases, the speed of hMSCs migration increases proportionally. Their analysis of hMSC proliferation on nanogrooved matrices, compared to flat ones, revealed no significant differences, hence concluding that proliferation of hMSCs may not be influenced by nanogrooves.

Recently, great deal of attention has been focused on electrospun nanofibrous for skin regeneration[74,80,83,84]. Using various nanotopographical designs, highly porous meshes of ultrafine fibers could be fabricated that closely resemble the nanotopography of the natural ECM of human skin[83]. Jin *et al*[85] investigated the *in*

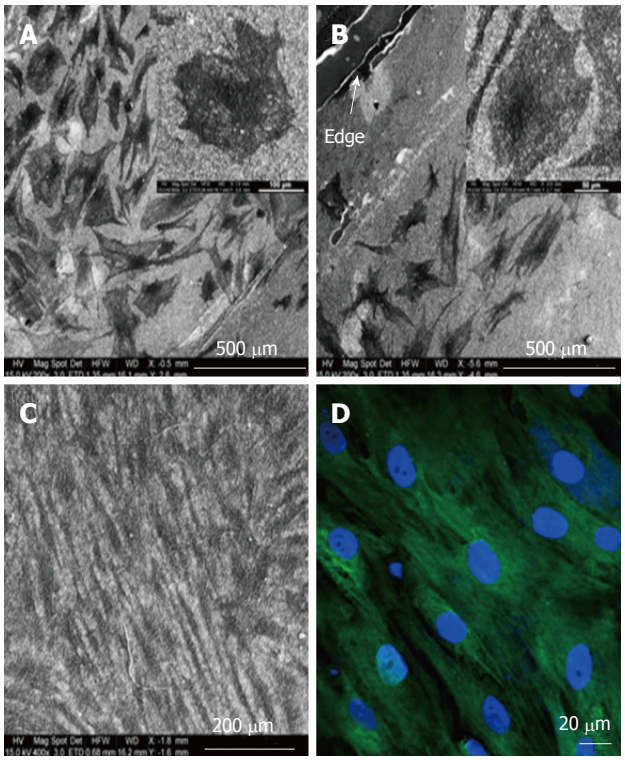

Figure 8 Electron microscopy images of differentiated and undifferentiated mesenchymal stem cells on Coll/PLLCL nanostructured nanofibrers. A: Mesenchymal stem cell (MSC) directed to differentiate along the epidermal lineage when cultured in epidermal induction medium; B: Epidermally differentiated MSC on the edge of a Coll/PLLCL scaffold (as shown by arrow); C: Electrospun Coll/PLLCL nanofibers seeded with undifferentiated MSC cultured in normal growth medium; D: MSC grown in normal growth medium on Coll/PLLCL nanofibers stained with Ker 10, after 15 d cell culture, imaged using laser scanning confocal microscope[85].

vitro differentiation potentials of bone marrow-derived MSCs to epidermal cells on electrospun collagen/poly (L-lactic acid)-copoly (3-caprolactone) (Coll/PLLCL) nanofibrous scaffolds[85]. To further mimic the structure of the natural ECM of human skin, they incorporated Coll/PLLCL nanofibrous scaffolds with collagen, at a ratio of 30:70, respectively. Their findings demonstrated that electrospun Coll/PLLCL nanofibers enhanced the level of MSC and scaffold interaction and that the electrospun scaffolds could mimic the native skin ECM. Furthermore, their results showed controlled and directed differentiation of MSCs along the epidermal lineage on Coll/PLLCL nanofibrous scaffolds (Figure 8), suggesting their potential use in skin TE applications.

POTENTIAL ADVERS EFFECTS OF ERODED NANOPARTICLES ON MSCS

Based on the evidence presented in this review, it is clear that nanotopography of a surface can have a great influence on the various cellular behaviours of MSCs including; their attachment, proliferation and most importantly their differentiation towards a specific lineage. However, based on the literature, there have been various reports of the adverse effects of nanoparticles on the cellular behaviour of MSCs as the result of implant erosion over-time[86-88]. This is especially a concern of biodegradable materials as they have been specifically designed to be deteriorated once implanted in their host. In long-term clinical application, the physiochemical properties of implants are influenced by their constant chemical, mechanical, and biological interactions with their host tissue and its surrounding environment[89]. These factors cause the degradation of the implant and subsequently to the release microscale and/or nanoscale wear particles in their immediate vicinity. These released particles no longer exhibit any of the nanotopographical characteristic of the implant surface, prior to implantation.

Released nanoparticles, once exposed to tissues and bodily fluids, tend to absorb macromolecules in their vicinity and depending on the surface characteristics of the nanoparticles (*e.g.,* their surface chemistry and surface energy), these macromolecules become attached onto the surface of the nanoparticles, leading to potential modification or functionalization of the surface of the nanoparticles[90,91]. Attachment of such macromolecules could change the affinity of a nanoparticle to bind with a specific protein, on the surface of a particular type of cells, which in return could have serious unaccounted for or adverse toxic effects on the proliferation and/or differentiation of cells[92]. For instance, there has been a report on serious DNA damage caused to MSCs when cultured with metallic silver nanoparticles, even at lower concentrations[87]. Calcium phosphate nanoparticles, very commonly used for bone tissue engineering applications, have also shown to affect MSC proliferation in a size-dependent manner, with larger particles causing more serious harm[86]. Furthermore, studies conducted by Hu *et al*[93] and Liu *et al*[88] showed that calcium phosphate nanoparticles could also affect MSC differentiation depending on their concentration and form of appearance. They reported that increasing the concentration of calcium phosphate nanoparticles, especially in the form of amorphous particles, rather than crystals, could negatively affect the osteogenic cell differentiation and matrix mineralisation of MSCs.

Other types of nanoparticles such as metallic ones are also of great concerns, as their release could lead to serious nanotoxicity in biomaterials and different cell lines. Various groups have investigated adverse systematic effects of titanium nanoparticles on different cell lines such as endothelial[94], lymphoblastoid[95], and fibroblasts[96] cells and reported of inflammatory reactions, DNA damage, and induction of apoptosis, respectively. However, until a year ago, there was no concise report on the adverse effect of titanium nanoparticles on the cellular behaviour of bone marrow-derived MSCs, despite titanium implants majorly being used for medical applications, such as in bone TE. As the result, very recently, Hou *et al*[97] investigated

Table 2 Significant studies on nanotopography and mesenchymal stem cells for developing 3D bone, cartilage and skin

Tissue/organ	Nanotopographical cues	Description	Outcome	Ref.
Bone	Nano-ridges, and nanogrooves nanotopography surfaces	The effect of nanotopographic ridges and grooves on MSCs morphology, proliferation and differentiation to osteoblast cells were investigated	Osteogenic differentiation can be controlled and directed using specific size scale of topographic cues with or without osteogenic agents	Watari *et al*[54]
	Implementing nanostructures of different sizes	The effect of titanium-coated hemisphere-like topographic nanostructures of various sizes (50, 100, and 200 nm) on hMSCs cellular behaviour towards osteoblast lineage was investigated	Osteogenic differentiation of hMSCs is dependent on the size of the underlying nanotopographical structures. Colloidal lithography combined with coating technologies can have great potentials for fabricating nanoscale topography on scaffolds	de Peppo *et al*[64]
	Bioactive calcium phosphate thin films sputter deposited onto a polycrystalline titanium nanostructured surface	Calcium phosphate thin films were used to study the cellular response of hMSCs to nanostructured titanium surfaces with the aim of directing them towards osteogenic differentiation	Various *in vitro* studies revealed that the use of nanostructured titanium surfaces and the bioactive calcium phosphate coatings could allow for directed and controlled differentiation of hMSCs towards osteogenic lineage. The combination of the two materials together showed higher rate of osteogenic differentiation compared to that of each of these materials on their own	McCafferty *et al*[63]
	Polished Ti surfaces chemically treated with H2SO4/H2O2 to create nanotopography	Chemically treated Ti surfaces with nanotopography and seeded with rat MSCs were used to investigate their osteoinductive potentials compared to untreated surfaces. Signalling pathways responsible for osteoinductive effect of nanotopography on MSCs were also investigated	Ti surfaces with nanotopography exhibited increased cell proliferation and alkaline phosphate activity. Gene expression of key bone markers was upregulated on nanotopography surfaces, under non-osteogenic conditions, compared to control	Rosa *et al*[66]
Cartilage	A biomimetic microfluidic device embedded with aligned nanofibers consisting of microchambers of different angle	A device was developed to create a microenvironment that integrates nanotopography and flow stimulus of the ECM of natural cartilage for the purpose of investigating the effect of microfluidic and nanotopography on the cellular behaviour and fibrochondrogenesis of MSCs	The angle of flow direction, in relation to the aligned nanofibers, affects MSCs behaviour. Fibrochondrogenesis of MSCs was evident when the flow direction was perpendicular to the aligned nanofibers	Zhang *et al*[75]
	Nanowire nanotopographic surfaces	Polycaprolactone nanowires surfaces were fabricated using a solvent-free gravimetric template technique to investigate their nanotopographical effects on the adhesion, proliferation, differentiation and ECM synthesis of adipose-derived MSCs	The results demonstrated that adhesion and proliferation of adipose-derived MSCs were enhanced on nanowire surfaces compared to the control. Nanowires also effected the morphology of these cells Interestingly, it was shown that nanowires supported adipogenic differentiation of these cells rather than chondrogenic differentiation	Trujillo *et al*[73]
	Nano-pillar, nano-hole and nano-grill nanotopography surfaces	Nano-pillar, nano-hole and nano-grill structures were formed on polycaprolactone surface using thermal nanoimprinting to investigate their effect on chondrogenic differentiation of hMSCs	Nanotopographical patterns have the ability to induce changes in MSC morphology and cytoskeletal structure towards a specific lineage, in this case chondrocyte cells. Delayed chondrogenesis was observed on nanogrill topography compared to nano-pillar and nano-hole topography, which enhance MSC chondrogenesis	Wu *et al*[55]
Skin	Electrospun nanofibrous scaffolds	Electrospun Coll/PLLCL and PLLCL nanofibrous scaffolds were prepared to investigate the proliferation and differentiation of MSCs to epidermal lineages	Cell proliferation was significantly higher on Coll/PLLCL nanofibrous scaffolds compared to PLLCL scaffolds. MSC morphology was also different on Coll/PLLCL nanofibrous scaffolds compared to control. Electrospun Coll/PLLCL exhibited similar properties to the native skin ECM	Jin *et al*[85]
	Nanotopographically variable grooved matrices	Nanotopographically variable grooved matrices, using UV assisted capillary force lithography, with curable PUA polymer were fabricated and then coated with gelatine to investigate the effect of nanotopographical density on hMSC migration and proliferation for wound healing purposes	As the density of the nanogrooved matrices increased, the speed of hMSCs migration increased proportionally. It was shown that hMSC proliferation was not significantly different on nanogrooved matrices, compared to flat control. Therefore, suggesting that proliferation of hMSCs may not be influenced by the nanogrooves	Kim *et al*[82]

PUA: Polyurethane acrylate; Coll/PLLCL: Collagen/poly(llactic acid)-co-poly(3-caprolactone); Ti: Titanium; hMSCs: Human mesenchymal stem cells.

the effects of titanium nanoparticles on adhesion, migration, proliferation and differentiation of MSCs and reported of serious negative effects of the nanoparticles on MSC migration as particle size increased. They

also demonstrated that exposure of MSCs to titanium nanoparticles negatively affected their osteogenic differentiation[97].

DISCUSSION AND FUTURE PROSPECTIVE

In this review, the role of nanotopography on controlling and directing cellular behaviour of various types of MSCs, with respect to specific tissues and organs, has been described. Based on the studies presented here, it can be established that various nanofabrication methods can be employed to design and fabricate nanostructured scaffolds with distinct nanotopographical cues to control and direct various cellular behaviours of MSCs including; their attachment, proliferation and most importantly their differentiation towards a specific lineage (Table 2). Therefore, incorporating nanotopography on the design of scaffolds would open doors to new generation of TE strategies for the development of functional organs and tissues.

The review of the literature demonstrated that most studies on this topic have been focused on bone TE applications. In most of these studies, such strategy seemed to improve the proliferation and differentiation of MSCs for repair or regeneration of bone. In most cases, authors have reported of controlled and directed osteogenesis of these cells on various polymeric based composite or nanocomposite scaffolds. However, there seems to be a clear lack of investigation into the potentials of using nanotopography for the development of more complex organs or tissues such as heart, kidney, or bladder. This is despite of the fact that some studies have confirmed the abilities of MSCs to differentiate into various other cell types; including muscle cells, stromal cells and fibroblast cells. Therefore, in the future, TE and regenerative medicine could greatly benefit from research focused on developing more complex organs or tissues using nanotopography guided differentiation of MSCs, while at the same time, there needs to be a more comprehensive investigation on the potential adverse effect of various types of nanoparticles, released from eroded nanotopographical surfaces, on the cellular behaviour of MSCs.

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