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REVIEW PAPER

Regulation of substrate surface topography on differentiation of mesenchymal stem cells

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Abstract
Mesenchymal stem cells (MSCs) have been extensively used in the field of tissue engineering and regenerative medicine. The effect of surface properties on the differentiation of MSCs is a very important issue for the design and fabrication of scaffolds or biomaterials. This review is mainly focused on the morphological or topographic characteristics of cell adhesion substrate, i.e. cell area and shape for individual cell, cell density and cell–cell contact for multiple cells, substrate roughness, ridge width, micropillar height, nanoparticle diameter and aspect ratio of nanowire. The results from different studies were quantitatively analyzed using comparable or unified parameters and definitions under the specific experimental conditions such as cell source, culture time, induction medium, matrix material and differentiation marker. Some interesting phenomena and properties were discovered by this integrated and systematic analysis, which might give insights into the regulatory mechanism of surface morphology or topography on MSCs differentiation.

Keywords Mesenchymal stem cell · Adhesion morphology · Surface topography · Differentiation · Biomaterial

1 Introduction

Mesenchymal stem cells (MSCs) are the only reminiscence of mesenchyme still present in adult organisms [1]. They have high capability of self-renewal and differentiation into multiple lineages, including neurons, myoblasts, osteoblasts, chondrocytes and so on. Because of the extensive differentiation capabilities, MSCs have been widely used as the candidates of seed cells in tissue engineering or regeneration medicine [2]. MSCs differentiation responds to both chemical and physical cues generated in the extracellular environment [3]. Since 1990s, the interaction between stem cells and biomaterials has been extensively investigated. It has been well recognized that extracellular microenvironment plays important roles in growth and differentiation of stem cells. Apart from soluble chemical factor in the extracellular solution [4] and chemical composition of the substrate where cells adhere to also gets involved with the differentiation of MSCs [5, 6], physical and geometric properties of substrate surface influence MSC differentiation. One milestone of the fundamental works on physical properties of substrate is the discovery in 2006 performed by Discher et al., i.e. that substrate stiffness significantly regulates MSCs differentiation [7]. In addition, a number of researches showed that the geometric factors such as intercellular connection [8, 9], cell shape [9–11], cell area [9, 12–14] and substrate morphology [15–19] are also able to modulate the differentiation of MSCs.

Along with the continuous progress of microfabrication technology, researchers fabricated simple periodic microstructures and nanostructures on the substrate surface to study the effects of these periodic topological microstructures on the physiological state of the cells, including roughness, ridge-groove, micropillar, nanoparticle or nanowire to mimic the in vivo adhesion microenvironment. In the present study, we summarized the researches published during the past 20 years and tried to quantitatively analyze the relation between these topographic parameters and MSC differentiation.

2 Adhesion morphology of individual MSCs

It is known that the terminally differentiated MSCs have specific shape, thus it is reasonable to examine whether MSCs

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with predominantly constrained shape are able to differentiate into the specific lineage. To clarify the mechanism how the adhesion morphology of individual MSCs influence their differentiation will be helpful to deeply understand the developmental process of biological tissues.

2.1 Cell shape

Since 1980s it has been found that the increase of spreading area of individual keratinocytes [20], hepatocytes [21], endothelial cells [22, 23] promoted cell growth but inhibited cell differentiation. After 2000s, MSCs were extensively adopted to study the relation between spreading area and osteogenic or adipogenic differentiation [12, 14]. When MSCs were cultured on circular or square patterns, along with the increase of spreading area, osteogenic differentiation was significantly increased but adipogenic differentiation was reduced (Fig. 1a, b). The spreading area of 170–400 μm^2, much less than the randomly cultured cell area of 1000 μm^2, led to 10–30% osteogenic differentiation but to 70–80% adipogenic differentiation of individual rat MSCs (rMSCs) after 7-days culture [9]. Higher spreading area up to 5000–6000 μm^2 significantly increased osteogenesis as 80% for rMSCs [9] or 40% for human MSCs (hMSCs) [13], and decreased adipogenesis as 18% for rMSCs [9] or 15% for hMSCs [13]. The above results suggest that when MSCs are constrained into small area by crowding or aggregating, adipogenesis may tend to occur, however the MSCs permitted to randomly spread might enter into osteogenesis.

Aspect ratio is defined as the ratio of maximal to minimal axis and is usually used as a typical geometric factor characterizing the rectangular adhesion shape of cells. When rMSCs were cultured onto 900 μm^2 rectangular micropatterns for 7 days, the decrease of aspect ratio from 8 to 1 did not reveal coincident tendency of osteogenesis (Fig. 1c) [24], but the adipogenesis is significantly promoted when decreasing aspect ratio from 16 to 1 (Fig. 1d) [11, 24]. When hMSCs were cultured on 2500 μm^2 rectangular micropatterns with aspect ratios of 1, 1.5 and 4 for 7 days [10], the results showed that the proportion of osteogenic differentiation decreased monotonously with the decrease of aspect ratio (Fig. 1c), while that of adipogenic differentiation had reversely trend (Fig. 1d). It should be noted that rMSCs with lower osteogenesis compared with hMSCs may be due to their smaller spreading area, additionally the adipogenesis for rMSCs is lower than hMSCs, which is independent of spreading area or aspect ratio.

Another geometric parameter to characterize the shape of individual cell is circularity ($= 4\pi \times Area/Perimeter^2$, Fig. 1e). Several studies adopted the flower-like, pentagonal and five-pointed star patterns with 2500 μm^2 to culture hMSCs [10], or used the circular, square, triangular and star-like patterns with same area of 900 μm^2 to culture rMSCs [11], or compared the circular, hexagonal, pentagonal, square and triangular patterns for hMSCs [25], or added different number of protrusions on circular pattern to seed hMSCs [26]. Combining the data of aspect ratio, higher circularity may inhibit the osteogenesis, but the results from hMSCs or rMSCs with the circularity around 0.7 show inconsistent tendency (Fig. 1c) [10, 24]. Compared with osteogenesis, the adipogenesis reveals more consistent relation with circularity, i.e. higher circularity promotes adipogenesis increasing with around 10–20% (Fig. 1d) [10, 11, 24–26]. Therefore, it is generally agreed that MSCs tend to be adipogenic for the higher circularity, whereas they are more likely to differentiate into osteoblasts for the lower circularity. But more systematic investigations should be performed to clarify the relation between osteogenic differentiation of MSCs and individual cells' shape.

It can be concluded from the above studies that the MSCs freely spreading on the surface of bone matrix with large area and low circularity may differentiate into osteoblasts but those MSCs with confined area and round shape tend to differentiate into adipocytes. It has been found that this phenomenon is regulated by the structure of cytoskeleton. For example, large and circular MSCs revealed well-organized stress fibers, while those cells confined to the small circular island had thicker stress fibers at cell edge than in the internal region [13]. In addition, cytoskeleton-related protein Ras homolog family member A (RhoA) got involved with the regulation of cell shape on the differentiation of stem cells, i.e. the activity of RhoA and Rho kinase (ROCK) was greater in the well-spread cells than the unspread ones, indicating that RhoA may be an ubiquitous integrator of both structural and soluble cues in developmental processes [12]. The above results suggest that the mechanical signals transmitted by cell shape could affect the lineage of stem cells through the interaction between cell shape, cytoskeleton mechanics and developmental processes.

2.2 Cell–cell connection

Besides the adhesion morphology of individual MSCs, the intercellular connection of multiple MSCs was demonstrated to influence cell differentiation. When increasing the density of MSCs, the establishment of cell–cell contact has been demonstrated to be promoted with the formation of multicellular clusters, which is stabilized by a fibronectin mesh [27]. Some research results showed that higher cell density or more cell–cell contact reduced osteogenesis but enhanced adipogenesis [9, 12, 28] (Fig. 2a, b). For example, for hMSCs plated at different densities of 1000–25,000 cells/cm^2 for 7 days or 28 days with both adipogenic and osteogenic induction medium, the cells with a large number of lipid droplets were found in high-density group, but the alkaline phosphatase (ALP), the osteogenic differentiation marker, was

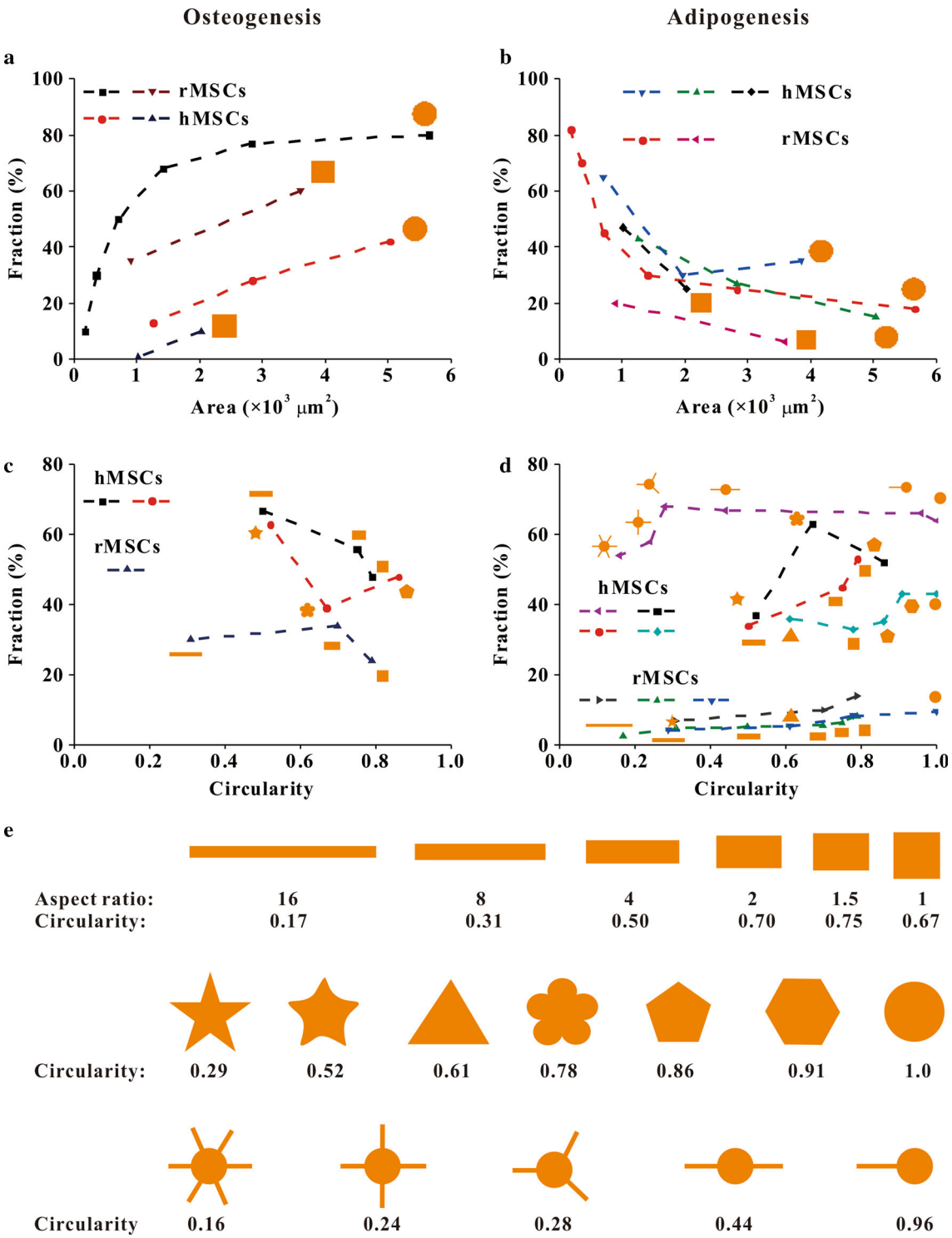

Fig. 1 Effect of spreading area and shape of individual MSCs on osteogenic or adipogenic differentiation. **a** Relation between spreading area and osteogenesis (redrawn from Refs. [9, 12, 13]). **b** Relation between spreading area and adipogenesis (redrawn from Refs. [9, 12, 13, 26]). **c** Relation between circularity and osteogenesis with constant area of 2500 μm^2 for hMSCs and 900 μm^2 for rMSCs (redrawn from Refs. [10, 24]). **d** Relation between circularity and adipogenesis with constant area of 900 μm^2 for rMSCs and 700 μm^2 to 2500 μm^2 for hMSCs (redrawn from Refs. [10, 11, 25, 26]). **e** The designed patterns and the corresponding values of circularity or aspect ratio. All MSCs in the above studies were cultured on the micropatterned surface for 7 days with induction medium

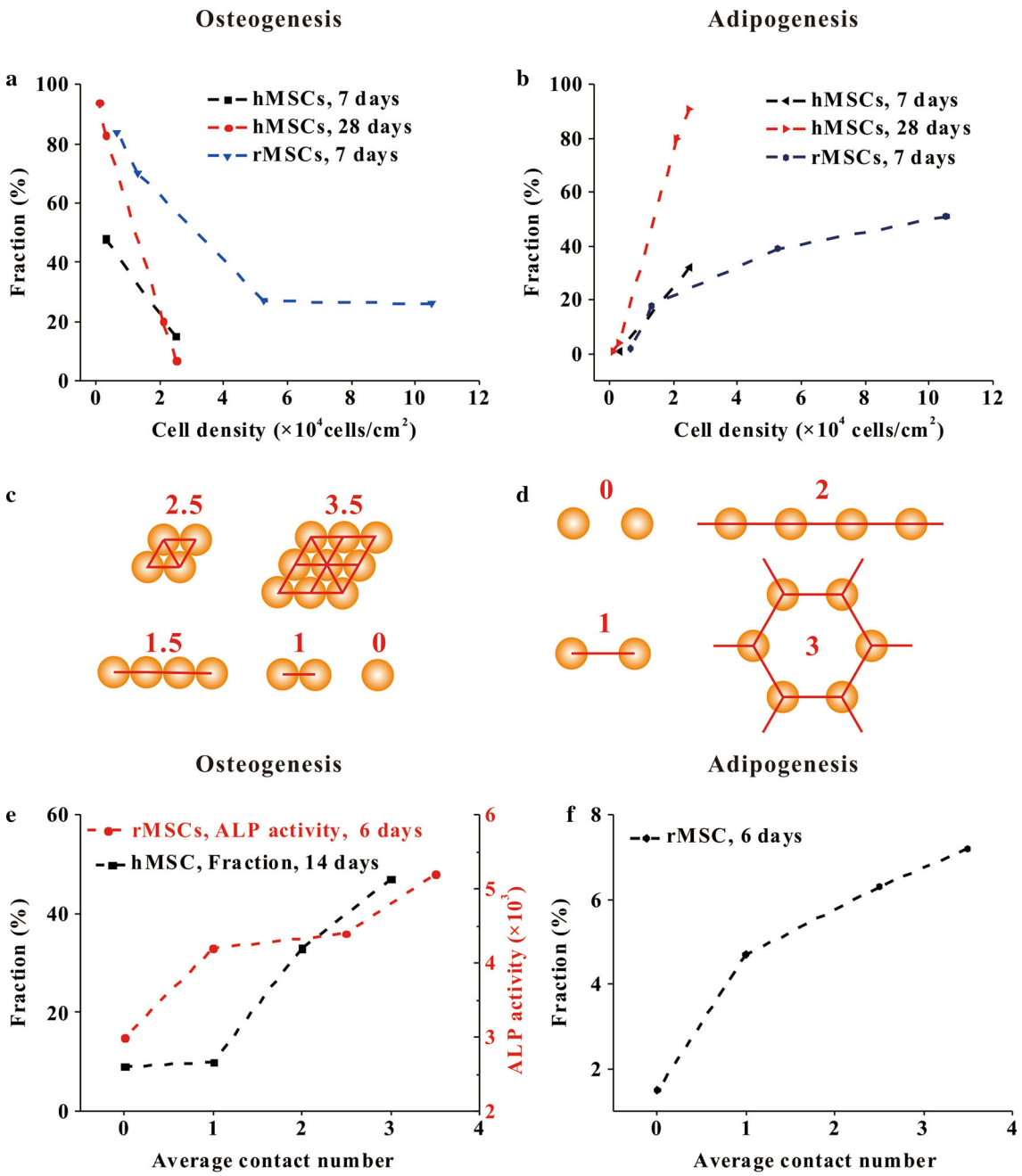

Fig. 2 Effect of cell density and cell–cell contact on osteogenic or adipogenic differentiation of MSCs. **a** Relation between cell density and osteogenesis (redrawn from Refs. [9, 12, 28]). **b** Relation between cell density and adipogenesis (redrawn from Refs. [9, 12]). **c** Patterns with different number of contacting islands [8]. **d** Patterned islands associated with each other by lines [30]. The red lines in **c** and **d** indicate the cell–cell contact and the red digits are the numbers of average contact. **e** Relation between average contact number and osteogenesis (redrawn from Refs. [8, 30]). **f** Relation between average contact number and adipogenesis (redrawn from Refs. [8, 31, 32])

more expressive in low-density group [12]. Similar results was found in rMSCs with higher cell density up to 1.05×10^5 cells/cm^2 for 7 days [9]. The above results revealed that when increasing cell density from 1000 to 1.05×10^5 cells/cm^2, the osteogenic proportion could decrease from 95% to 20%, while the adipogenic proportion could increase from 1% to 51%. It is reasonable to conclude that increasing cell density may reduce their spreading area, thus the above relation between cell density and differentiation is similar to the results on the effect of cell area on the differentiation of individual cells. Contrast to 2D-cultrue results, however, when MSCs were cultured in 3D matrix, their osteogenic potential was enhanced but adipogenic potential was repressed with increasing 3D accumulation level [27, 29]. The intrin-

sic mechanism of different lineage commitment of MSCs in 2D or 3D culture condition is still unknown and needs to be clarified.

It should be noted that when increasing cell density, cell area becomes smaller and cell–cell contact is promoted [27]. To identify the separated contribution of cell–cell contact on cell differentiation, the micropatterned circular islands associated with each other by different average contacting number of 0, 1, 1.5, 2.5 and 3.5 were designed and fabricated (Fig. 2c) [10]. After rMSCs were cultured on these patterns with osteogenic or adipogenic medium for 6 days, either osteogenic or adipogenic differentiation was promoted along with the increase of average contacting number (Fig. 2e, f). To strictly control the contact number per island, Wang et al. designed another pattern in which each circular island was associated with constant numbers 0, 1, 2 and 3 of neighboring islands through the lines with the length 30 μm and the width 2 μm (Fig. 2d) [30]. When hMSC was cultured on the patterns with osteogenic medium for 14 days, the osteogenic differentiation was significantly enhanced along with the increase of contacting number for each cell (Fig. 2e). The above studies suggest that more gap junctions might be formed when increasing intercellular contact so as to allow the direct transmission of more signal factors and metabolites between adjacent cells, which finally regulate cell differentiation.

The above results showed that cell–cell connection could promote both osteogenic or adipogenic differentiation of MSCs (Fig. 2). The fluorescent images of F-actin exhibited that MSCs with cell–cell interaction exhibited concentrated stress fibers at the connecting line [30]. This was consistent with the conclusion that cell–cell contact could regulate cytoskeletal tension through adhesion junctions and alter actin structure via cadherin. In addition, intercellular junctions also allowed direct transmission of signaling molecules and metabolites to adjacent cells, which may also be an important factor in the regulation of differentiation. However, further researches still need to be performed for clarifying the mechanism that either osteogenesis or adipogenesis of MSCs was enhanced by cell–cell connections.

3 Effect of substrate topography on the differentiation of MSCs

3.1 Roughness

Surface roughness of implant or scaffold is an important parameter to control the in vivo integration in tissue regeneration or tissue engineering. For example, it is believed that surface roughness increases the surface area of the implant, allowing more initial substrate deposition and early bone growth [33]. The average roughness (R_a) has been generally

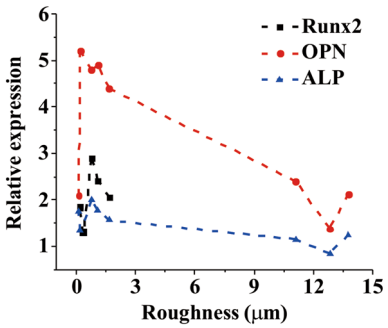

Fig. 3 Effect of roughness on osteogenic differentiation of MSCs (redrawn from Refs. [5, 31–34])

adopted to characterize the surface roughness of biomaterials and is defined as

$$R_a = \frac{1}{n \cdot m} \sum_{x=1}^{n} \sum_{y=1}^{m} [z(x, y) - \bar{z}], \quad (1)$$

in which the surface height $z(x, y)$ are measured at $n \times m$ sample points in x–y plane and $\bar{z}$ denotes the average value of $z(x, y)$. We normalized the gene expression in MSCs cultured on a plate surface with specific roughness relative to that for control group, i.e. the blank culture plate of polystyrene or hydroxyapatite. This relative expression of differentiation markers of ALP, runt-related transcription factor 2 (Runx2) and osteopontin (OPN) from several studies was summarized in Fig. 3. The experimental details for these studies can be found in Table 1.

For the roughness ranging from 120 to 1000 nm, the increase of substrate roughness promoted osteogenic differentiation of rMSCs [34] and hMSCs [5]. Briefly, the 3D porous graphene nanosheets synthesized on the titanium surface enhanced ALP activity, extracellular matrix mineralization and collagen secretion [34]. In addition, the polyurethane biomaterials with roughness 324–253 nm showed a significant increase of osteogenesis [5]. For the roughness larger than 1 μm, the expression of three osteogenic markers was significantly reduced when increasing substrate roughness for hMSCs on hydroxyapatite [31] or on polycaprolactone [33] or for rMSCs on titanium [32]. The above results indicate that the substrate roughness around 1 μm might be suitable for osteogenic differentiation of MSCs.

Some studies have confirmed that the surface roughness of extracellular substrates could affect the expression of osteogenic markers of stem cells (Fig. 3). It has been found that surface roughness altered the adhesion state and geometric shape of cells [5, 31]. The cytoskeleton of MSCs showed higher tension on the rougher surface by sensing the roughness gradient, which was further transferred to the nucleus and may regulate the expression of YAP/TAZ. Cytoskeletal filaments have been shown to pass through

Table 1 Summary of MSC differentiation regulated by substrate roughness

Cell	Induction medium	Time (days)	Material	Roughness (R_a, μm)	Differentiation markers	Conclusion	References
rMSC	–	1, 4, 7	Graphene nanosheets-modified titanium	0.122	OPN, ALP, BMP-2, OCN, mineralization nodules	OD	[34]
hMSC	OM	7, 14	Polyurethanes	0.324–0.253	ALP, mineralization nodules	OD	[5]
hMSC	OM	7, 21	Hydroxyapatite	0.2–1.65	ALP, mineralization nodules, Runx2, osteogenesis marker genes	0.77 μm or 1.09 μm promotes OD	[31]
hMSC	OM	1, 4, 7, 10, 14, 21	Polycaprolactone	0.5–4.7	ALP, COL I, mineralization nodules	2.1–3.1 μm promotes OD	[33]
rMSC	–	7	H_2O_2-Ti, NaOH-Ti, HCl-Ti	12.818, 11.090, 13.765	ALP, OCN, OPN	OD	[32]

OM osteogenic medium, *OD* osteogenic differentiation, *BMP-2* bone morphogenetic protein 2, *OCN* osteocalcin, *COL I* type I collagen)

nuclear pores and connect with nuclear scaffolds, which may provide the pathways for mechanical signaling transduction. In addition to roughness, chemical properties of substrate materials may also influence cell behavior. For example, HCl-Ti substrate significantly enhanced cell attachment, and graphene nanosheets-modified titanium substrate enhanced the osteogenic differentiation of rBMSCs due to its superior protein adsorption capacity [32, 34].

3.2 Ridge-groove

To avoid the disadvantage that roughness is difficult to be characterized, the substrate with ordered surface topography, e.g. the ridge-groove structure with the scale of nanometer or submicron (Fig. 4a), was found to have a strong influence on osteogenic differentiation and to increase mineral deposition [35]. The ridge width has been proved to play an important role in regulating the lineage commitment of MSCs and the experimental details for several studies can be found in Table 2. Watari et al. found that the grooved substrate with the ridge width of 0.2 μm is suitable for the osteogenesis of hMSCs compared with the ridge width of 0.7 μm or 2 μm, regardless of the presence or absence of osteogenic induction medium (Fig. 4b) [36]. Abagnale et al. gave a similar conclusion that hMSCs cultured on the surface with smaller ridge width of 2 μm for 14 days tended to osteogenic differentiation compared with 3–15 μm ridge width, while the groove width did not have significant influence [37]. When the ridge width is further increased to be more than 20 μm, however, the higher ridge width of 30–180 μm gradually promoted

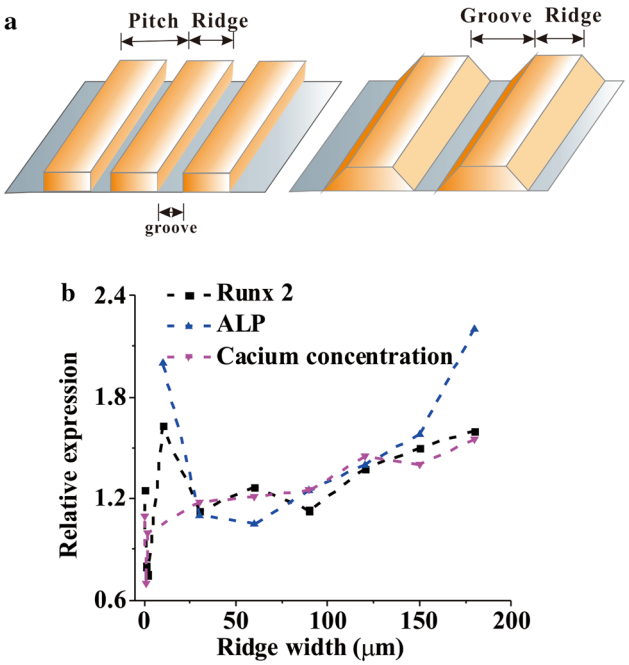

Fig. 4 Structure of ridge-groove substrates and its effect on osteogenesis of MSCs. **a** The definition of structure parameters for ridge-groove substrate. **b** The regulation of ridge width on osteogenic differentiation (Redrawn from Refs. [35–37, 39, 40])

the osteogenesis of hMSCs [38]. The above results imply that the ridge-groove substrate with about 10 μm of ridge width, similar to the cell size, or with the ridge width more than 150 μm, might be suitable for osteogenic differentiation of MSCs.

Table 2 Summary of MSC differentiation regulated by ridge-groove

Cell	Induction medium	Time (days)	Material	Pitch (μm)	Ridge (μm)	Height (μm)	Groove (μm)	Differentiation markers	Conclusion	References
hMSC	OM	7, 14, 21	Polyurethane	0.4, 1.4, 4	0.2	0.3	–	Runx2, calcium deposition	400 nm promotes OD	[36]
					0.7					
					2					
hMSC	OM, AM	14	Polyimide	–	15	2	–	Mineralized nodule, lipid droplets	AD	[32]
				–	2	2	–		OD	
				0.65		0.2	–		AD and OD	
hMSC	–	3	Polyimide	–	10	10	10	ALP, Runx 2, COL I, LPL	Promotes OD, inhibits AD	[37]
hMSC	OM	7, 14, 28	Ti	–	60	10	60	ALP, calcium deposition, mineralized nodule	OD	[40]
hMSC	OM	14, 21	Alumina ceramic	–	180	–	180	Calcium deposition, ALP, Runx2, OPN, OCN	OD	[38]
rMSC	–	28–56, in vivo	Cylindrical epoxy resin implants		0.15	0.05	0.15	Bone volume	800 nm or 200 nm promotes OD	[35]
					0.8	0.07	0.2			

AM adipogenic medium, *OD* osteogenic differentiation

More evidences indicated that the transmembrane protein integrin gets involved the regulation of ridge-groove structure of substrate on osteogenic differentiation of stem cells. Integrin is usually regarded as a key mediator of mechanotransduction for extracellular signals. Cells could sense the morphology features of substrate surface through the aggregation of integrins and the formation of focal adhesions [36, 37]. The size of ridge had a direct effect on the morphology of MSCs, in which the elongated cells tended to be in osteogenesis, while the restricted spreading area promoted adipogenic differentiation.

3.3 Micropillar

Micropillar is one-dimensional structure perpendicular to substrate surface and is also usually adopted to study cell-material interaction. The experimental details and conclusions for some studies can be found in Table 3. Pan et al. cultured rMSCs on square micropillar with side length 3 μm and different heights of 0.2 μm, 1 μm or 5 μm for 10 days [41]. They found that the 5 μm-height pillar could severely deform the nucleus, which was inserted into the micropillars, but rMSCs still have the ability of osteogenic differentiation. The authors further studied the effect of micropillar height on the differentiation of rMSCs (Fig. 5a) [42]. The results showed that the higher micropillar of 4.6 μm or 6.4 μm promoted osteogenic differentiation, while the lower micropillar of 0.8 μm enhanced adipogenic differentiation. Some other studies demonstrated that the osteogenic differentiation of hMSCs cultured on 3 μm- [43] or 100 μm-height [44] micropillars was promoted compared with those on blank surface. However, Konttinen et al. found 5 μm-height square pillar might be best substrate for osteogenic differentiation of hMSCs compared with 0.2 μm- or 20 μm-height pillar [45]. These research results revealed that micropillar structure with large height might be better for osteogenic differentiation but more systematic investigation is still needed to be performed.

It has been found that the micropillar exceeding a critical height can significantly deform the nucleus, which may further cause the differentiation of stem cells. The micropillar structure reduces the projected area of cells and reduces cell tension, which may be similar to the adherent characters of MSCs on bone surface. The experimental observation showed that MSCs on a smooth substrate had a small amount of focal adhesions, while those cultured on the micropillar substrate displayed more dot-like vinculin structure, revealing that the microstructural feature could make more interactions between cells and substrate [41–43]. Moreover, the lower micropillar height leads to cytoskeleton tension, enhances the expression of ERK, and ultimately regulates bone formation [45].

Table 3 Summary of MSC differentiation regulated by micropillars

Cell	Induction medium	Time (days)	Material	Side-length (μm)	Gap size (μm)	Height (μm)	Differentiation markers	Conclusion	References
rMSCs	OM	10	PLGA	3	6	0.2, 1, 5	ALP	OD	[41]
rMSC	OM, AM	7	Poly(lactide-co-glycolide)	3	–	0.8, 3.2, 4.6, 5.3, 6.4	ALP, Runx2	4.6 or 6.4 μm promotes OD	[42]
							LPL, PPARγ	0.8 μm promotes AD	
hMSC	–	21	SiO_2	5	10	3	ALP, COL1, Runx2, BMP2.	OD	[43]
hMSC	OM	14, 21	Hybrid polymer	100	100	0.2, 5, 20	ALP,OPN, mineralized nodule	Lower pillar promotes OD	[45]
hMSC	–	21	PDMS	100	–	100	ALP, COL1, Runx2,OCN	OD	[44]

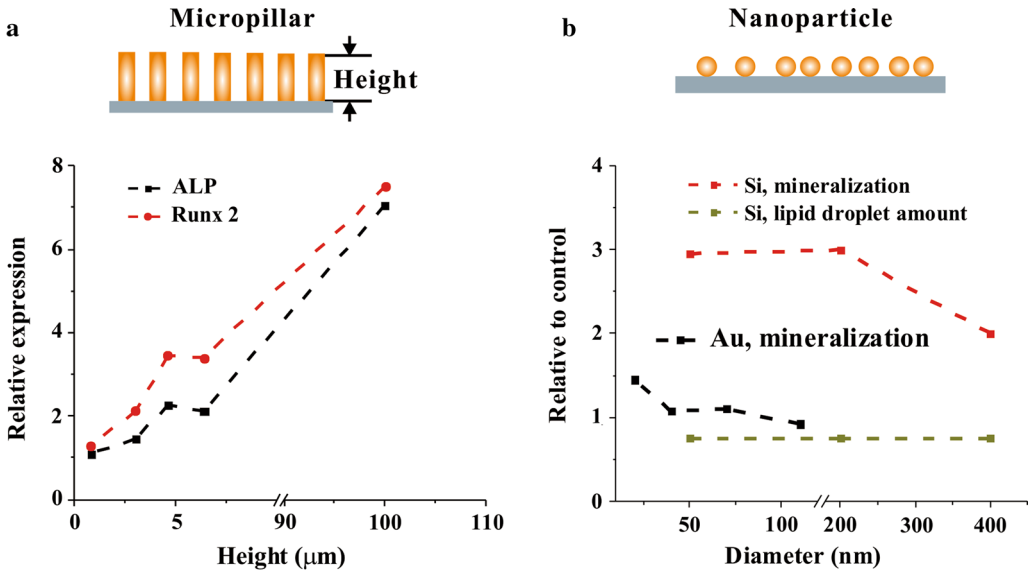

Fig. 5 Regulation of micropillar or nanoparticle substrate on the differentiation of MSCs. **a** Effect of micropillar's height on the relative expression of osteogenic markers (redrawn from Refs. [42–44]). **b** Effect of nanoparticles' size on the differentiation of MSCs (redrawn from Refs. [46–50])

3.4 Nanoparticle

There has been increasing evidence that nanomaterials can promote stem cell therapy and bone tissue engineering. The experimental details and conclusions for some studies can be found in Table 4. It has been confirmed that gold nanoparticles in sphere with 40 nm or 70 nm diameter and in rod with 70 nm diameter promoted osteogenic differentiation and inhibited adipogenic differentiation of hMSCs, in which the smaller gold nanoparticles lead to higher level of osteogenesis (Fig. 5b) [46, 47]. However, the rod-like nanoparticle with 40 nm diameter inhibited osteogenic differentiation. Yi et al. also found that 20 nm-gold nanoparticles upregulated the expression of osteogenic markers or mineralized nodule formation and inhibited adipogenic markers of rMSCs compared with NaF control substrate [48]. Similar to gold nanoparticles, silicon nanoparticles of 50 nm, 200 nm or 400 nm significantly promoted the expression of osteogenic markers and mineralization of hMSCs compared with control group, in which 50 nm- or 200 nm-particles had significantly higher osteogenesis than 400 nm-particles [49]. The results for hMSCs on silicon nanoparticles with same size showed that the formation of lipid droplets in the cytoplasm was less than those in the control group

Table 4 Summary of MSC differentiation regulated by nanoparticle

Cell	Induction medium	Time (days)	Nanoparticles	Size (nm)	Differentiation markers	Conclusion	References
hMSC	OM	14, 21	Au-PEG-TEMPO NPs	40	ALP, Runx2, SPP1, calcium deposition	OD	[47]
	AM				Lipid droplets, LPL	Inhibits AD	
hMSC	OM	7, 14, 21	Au NPs	40, 70,110 (sphere); 40,70,110 (star); 40, 70, 110 (rod)	ALP, Runx2, calcium deposition	40 nm, 70 nm (sphere) and 70 nm (rod) promote OD 40 nm (rod) inhibits OD	[46]
rMSC	OM	7, 10, 14	Au NPs	20	ALP, mineralization nodules, osteogenesis marker genes	OD	[48]
	AM				Lipid droplets, adipogenic marker genes.	Inhibits AD	
hMSC	–	1	PVP-coated Ag NPs	30	Adiponectin secretion, lipid droplets, adipogenic marker genes	Not affect AD	[57]
hMSC	OM	14, 21	Silica	50, 200, 400	ALP, mineralization nodules	OD	[49]
hMSC	AM	14	Silica	50, 200, 400	Lipid droplets, adiponectin, PPARγ, C/EBPα	Inhibits AD	[50]
hMSC	OM	7, 10, 14	Iron oxide	30	ALP, Runx2, mineralization nodules	OD	[51]
hMSC	OM	8, 21	Hydroxyapatite	98	ALP, IBSP, OCN, DCN	OD	[52]

NPs nanoparticles, *SPP1* secreted phosphoprotein 1, *LPL* lipoprotein lipase, *IBSP* integrin binding sialoprotein, *DCN* decorin

[50]. Moreover, iron oxide nanoparticles of 30 nm [51] or hydroxyapatite nanoparticles of 98 nm [52] have been shown to promote osteogenic differentiation of hMSCs. These results indicate that the nanoparticles could promote osteogenic differentiation but inhibit adipogenic differentiation.

The regulation of nanoparticles on MSCs differentiation is mainly through the typical signaling pathways of mechanotransduction. For instance, gold or iron oxide nanoparticles generated mechanical stress in MSCs and activated p38 mitogen-activated protein kinase (MAPK) pathway, which led to the upregulation of osteogenic genes and the downregulation of adipogenesis-specific genes [48, 51]. The nanaoparticles may be internalized into MSCs and induce the formation of actin stress fiber to stimulate osteogenesis as well as reduce adipogenesis [49]. The rigidity of nanoparticles is closely related to their translocation ability through cell membrane. The stiffer nanoparticles achieved a higher cellular uptake efficiency because they had full internalization by forming a complete double-layer endosome coating, while relatively soft nanoparticles only reached 40% surface coverage by membrane lipids. Whether the absorption rate of nanoparticles has a significant effect on the differentiation degree of stem cells still needs to be investigated [53].

3.5 Nanowire

Silicon nanowires are easily chemically modified to obtain controlled surface properties and their size is comparable to that of biomolecules, therefore they are used as scaffold materials in tissue engineering. The experimental details and conclusions for some studies can be found in Table 5. For the hMSCs cultured on the substrate aligned with silicon nanowire, high level of osteogenic differentiation was observed in the nanowire with the smaller aspect ratio of 54 (8.7 μm:162 nm) compared with other long nanowire (Fig. 6a) [54]. Another study confirmed that hydroxyapatite in porous ceramics with an aspect ratio of 46 (17.5 μm:380 nm) could regulate rMSCs to differentiate into the osteogenic lineage [55]. When rMSCs were cultured on the substrate with vertical array of TiO_2 nanotubes, whose diameter influenced the cell fate, i.e. 15-nm nanotube significantly promoted the osteogenic differentiation compared with those on nanotube surface with larger diameter (Fig. 6b) [56]. Therefore, these results suggest that the nanowire substrate with smaller aspect ratio or the nanotube substrate with smaller diameter might direct the osteogenic lineage commitment of MSCs. The silicon nanowires with smaller aspect ratio have higher spring constants induces cytoskeleton remodeling by activating focal adhesion kinase through

Table 5 Summary of MSC differentiation regulated by nanowires or nanotubes

Cell	Time (days)	Material	Length (μm)	Diameter (nm)	Aspect ratios (diameter:length)	Differentiation markers	Conclusion	References
hMSC	1, 3	Silicon nanowires	4.5	150	1:30	COL I, Runx2, β-III tubulin, nestin	Promotes neuron-like differentiation, inhibits OD	[58]
hMSC	3	Silicon nanowires	8.73	162.3	1:54	COL I, Runx2	162.3 nm promotes OD	[54]
			13.50	170.6	1:79			
			20.18	174.7	1:115			
			25.93	191.7	1:135			
rMSC	4, 14	Hydroxyapatite nanowire	17.5	380	1:46	BMP-2, OPN, OCN	OD	[55]
rMSC	14	TiO_2 nanotube		15, 20, 30, 50, 70, 100		Mineralized nodule, OCN	OD	[56]

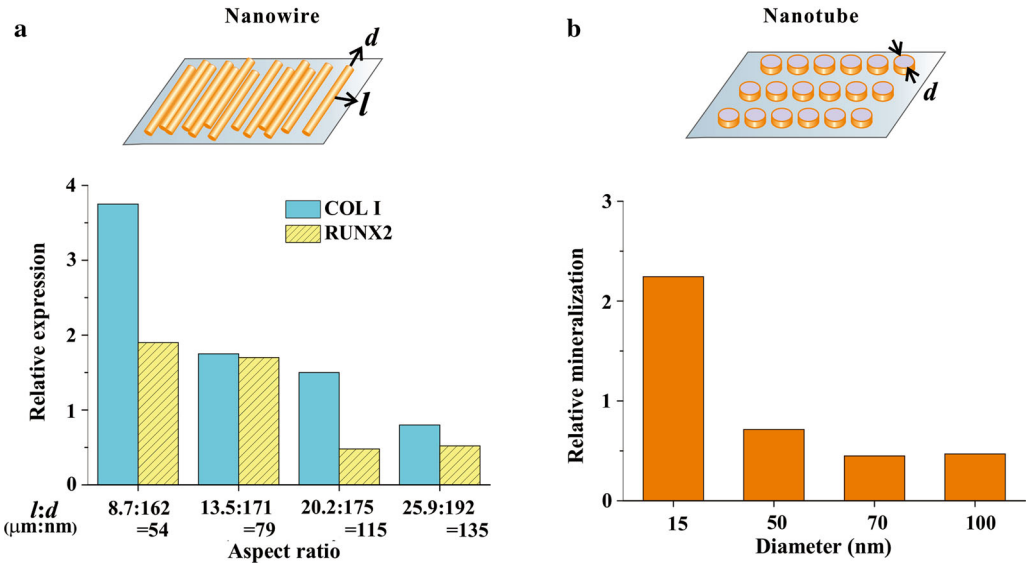

Fig. 6 Regulation of nanowire or nanotube on the differentiation of MSCs. **a** Effect of nanowires' aspect ratio on the relative expression of osteogenic markers (redrawn from Refs. [54, 55]). **b** Effect of nanotubes' diameter on the relative mineralization (redrawn from Ref. [56])

integrin-extracellular matrix interaction and finally enhances osteogenic differentiation [54].

4 Conclusion

In this review, we quantitatively summarized the results about the relation between the differentiation of MSCs and the morphological or topographical parameters of substrate. Regardless of the complex experimental conditions in different studies or groups, some interesting phenomena or properties have been given from this integrated and systematic analysis. The larger spreading area of individual cell, lower cell density or more cell–cell contact, higher micropillar, shorter nanowire and optimal roughness or ridge width might promote the osteogenic lineage commitment of MSCs. Cell shape (e.g. circularity of individual cell) or particle diameter also plays important role in regulating cell differentiation of MSCs, but the investigation considering more factors such as cell source, chemical composition of substrate should be performed in the future. We believe that this analysis could provide new insights into the regulatory mechanism of surface properties on the lineage commitment of MSCs and be helpful for guiding the design and fabrication of the scaffold in tissue engineering.

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Compliance with ethical standards

Conflict of interest We don't have conflicts of interest.

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