JPhys Materials

PAPER

Effect of laser induced topography with moderate stiffness on human mesenchymal stem cell behavior

Chenyuan Gao\textsuperscript{1,2,3}, Lin Tang\textsuperscript{4}, Jieyu Hong\textsuperscript{5}, Chunyong Liang\textsuperscript{3}, Lay Poh Tan\textsuperscript{4} and Huaqiong Li\textsuperscript{1,2,3} \textsuperscript{\textcopyright}

\textsuperscript{1} Key Laboratory of Orthopaedics of Zhejiang Province, The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, 325035, People’s Republic of China
\textsuperscript{2} School of Biomedical Engineering, School of Ophthalmology & Optometry and Eye Hospital, Wenzhou Medical University, Wenzhou, Zhejiang Province, 325035, People’s Republic of China
\textsuperscript{3} Engineering Research Center of Clinical Functional Materials and Diagnosis & Treatment Devices of Zhejiang Province, Wenzhou Institute of Biomaterials and Engineering, Wenzhou, Zhejiang Province, 325011, People’s Republic of China
\textsuperscript{4} School of Materials Science & Engineering, Nanyang Technological University, 639798, Singapore
\textsuperscript{5} School of Materials Science and Engineering, Hebei University of Technology, Tianjin 300130, People’s Republic of China

E-mail: lihq@wibe.ac.cn

Keywords: stem cells, differentiation, scaffold properties

Abstract

Though the effects of scaffold properties such as stiffness and topography on stem cell behavior was well known of, there were multiple theories that explain such behavior and there has been no common consensus thus far. This study deals with using polydimethylsiloxane (PDMS) to mimic a specific microenvironment that favors human mesenchymal stem cells (hMSCs) differentiation into myogenic lineages through the manipulation of specific surface topography and appropriate substrate stiffness. Femtosecond laser was applied to machine microchannels on PDMS substrates in this work. hMSCs were seeded and cultured on lasercut substrates, uncut substrates and controls. Quantitative and qualitative analyses of stem cell behavior were discussed herein with the use of Picogreen Assay for cell proliferation, staining of cytoskeleton for cell orientation, immunostaining of Myosin Heavy Chain for a myogenic biomarker, and quantitative real time polymerase chain reaction for gene expression analysis. It was found that both myogenic differentiation of hMSCs could be achieved by moderate stiffness or microchannels. And differentiation was further boosted by such PDMS substrates with additional microchannels.

1. Introduction

Ever since the discovery of stem cells’ multipotency in the 1860s, bone marrow derived human mesenchymal stem cells (hMSCs) have been widely adopted in the field of tissue engineering researches\textsuperscript{[1, 2]}. Like all other stem cells, hMSCs could differentiate into various cell types including myoblasts\textsuperscript{[3]}, osteoblasts\textsuperscript{[4]} and neuroblasts\textsuperscript{[5]}. As cells work by nature, they could neither be manipulated nor instructed to exhibit cell behavior in a certain way. Tissue engineers work to mimic cell environments in the body, trick cells to recognize these artificial microenvironments, widely known as scaffold\textsuperscript{[6, 7]}, and differentiate accordingly as they stay in the body\textsuperscript{[8–10]}. Besides cell differentiation, scaffolds properties are also known to have effects on other cell behavior such as proliferation, adhesion and morphology\textsuperscript{[11]}. It is thus crucial to study the effect of scaffold properties on hMSCs behavior, to guide cell growth as far as possible on their path to the eventual desired cell fate or tissue regenerative purposes. Scaffold properties could be sub-divided into physical and surface chemical properties, all of which play major roles in directing stem cell growth\textsuperscript{[12, 13]}. Physical properties such as shape, topography, stiffness and porosity has shown their potentials on controlling stem cell behavior\textsuperscript{[14]}. Topography, particularly controllable micro or nanoscale topography, is one of the most important physical cues, which has been demonstrated to exhibit its great guidance on cell behavior, even in the absence of any biochemical induction medium\textsuperscript{[15]}. Lately, as laser technology has progressed, the modification of materials using lasers has attracted much attention from many scholars. All kinds of materials can be modified by laser
machining together with varieties of applications [16, 17]. Laser technology has many advantages over traditional technology, such as scalability and it can be easy to operate, it does not need any templates. Also it can be carried out rapidly even under ambient conditions. Femtosecond laser is one of the fast laser type that can reduce the area of the heat-affected zone compared to lasers with a longer pulse width. It has been widely applied for the surface modification of polymers [18]. The application of femtosecond laser machining in biomaterial is still very limited, even though this method is highly efficient in creating unique surfaces and patterns. In our previous work, we too have utilized direct femtosecond laser machining to create microgrooves on the scaffolds and investigate the effects of topography on cell growth [19–21]. The optimized microchannels (with 30 μm width, 30 μm pitch and 20 μm depth) were achieved to induce stem cell alignment and myogenic differentiation without addition of any inductive factors. The possible molecular mechanism of topography induced cell commitment was also documented [22]. Topography induced cytoskeleton reorganization through force-mediated proteins has been argued to be the key driver of this process. Notably, vinculin is one of the widely studied force-sensitive proteins, which could regulate stress fiber and focal adhesion formation, then lead to changes of cell shape and intracellular signal through FAK-ERK or Rho-ROCK pathways [22–24]. Furthermore, it has been proved that matrix with different stiffness (soft, intermediately stiff and hard) which mimic the stiffness of different tissues (brain, muscle and bone) could regulate stem cell specific differentiation towards corresponding tissue [25]. hMSCs cultured on the substrate with intermediately stiff (range from 8 to 17 kPa) perform an elongated cell shape at a relatively high-tension state, which promote the myogenic commitment of stem cells. The resulted cell signaling and differentiation might due to extracellular matrix (ECM) stiffness induced integrin recruiting, myosin-based contractility and stress fiber reorientation [26]. Both topography and mechanical property of ECM might therefore affect mechanotransduction, physical cues induced signaling, via cytoskeleton reconstruction.

Thus, in this study, we used the direct femtosecond laser machining to create highly uniform microchannels (with 30 μm width, 30 μm pitch and 20 μm depth) directly on polydimethylsiloxane (PDMS) substrate with moderate stiffness (around 10 kPa), to investigate the combined effect of matrix compliance and topography on stem cell behavior without any exogenously induction medium. We hypothesis that optimized matrix stiffness combined with microchannels could largely coax cell orientation and enhanced myogenic differentiation might be achieved. This study will provide an insight on considering both matrix mechanical and topographic properties, using an efficient laser machining, when designing engineered scaffold for the possible clinical muscle tissue regenerations.

2. Materials and methods

2.1. Preparation of PDMS film

A mixture of PDMS base and curing agent from Sylgard 184 Silicone Elastomer Kit[x] were prepared as instructed at 50:1 weight proportion (8 g:0.16 g) and mixed well over a 10 min time frame. The mixture was then casted on 22 mm by 22 mm glass cover slips using film applicator and casting knife adjusted to 50 μm height. Casted films were first degassed in 60 °C vacuum oven for 30 min before being transferred to normal 60 °C oven to cure overnight. This was to prevent bubble formation at or near the film surface due to the naturally high viscosity of PDMS. After curing, the samples were washed overnight with methanol to remove uncured precursors. Washed samples were dried in the oven and stored well in a desiccator prior to further processing steps.

2.2. Femtosecond laser machining

As shown in the following scheme 1, while half of the uncut fabricated PDMS samples were ready for surface modification, the other half were gifted with 20 mm by 20 mm area of 30 μm width by 20 μm depth using previously defined femtosecond laser conditions of 2 scan passes at 75 mW laser power and 10 mm s⁻¹ scan speed, using a Ti-sapphire (Clark-MXR CPA 2001, Dexter, MI) infrared solid-state laser.

2.3. Surface modification

The lasercut and uncut PDMS samples were first treated with oxygen plasma at 120 mTorr for 60 s. 40 μl APTES and 460 ml EtOH in DI water were added to the treated samples and left to react for 40 min. Samples were then washed with 100% EtOH for 5 min and dried in 60 °C oven for an hour. Dried samples retrieved from the oven were cooled to room temperature before adding light-sensitive GA to them and left in the dark for an hour. GA was removed, and samples were washed with deionized water thrice for 30 min for thorough removal of unreacted GA. It is important for complete removal of GA as it is toxic. After washing, samples were first settled in PBS for 5 min before collagen Type I was coated carefully and left overnight. Excess collagen was carefully
removed the following day before the samples were soaked in PBS (to prevent samples from drying up) and stored in 4 °C fridge prior to cell culture.

2.4. Sample characterization
To confirm that collagen type I was successfully coated on the PDMS substrates, the composition of unmodified PDMS samples and surface modified samples were characterized by Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy (PerkinElmer Spectrum GX spectrometer) range from 600 to 4000 cm⁻¹, where diamond crystal was used as the internal reflection element. The average values of all spectra are calculated by eight scans with a resolution of 2 cm⁻¹, and the atmospheric water and carbon dioxide are corrected.

2.5. Cell culture
hMSCs was acquired from Lonza (Cambrex, UK), and cultured as previously [20–22]. As provided by the vendor in the user manual, cells expressed CD105+/−, CD166+/−, CD29+/−, CD44+/−, CD14−/−, CD34−/−, and CD45−/−. Cells from passage 4 or 5 were seeded at seeding density of 4000 cells cm⁻² and cultured in low glucose Dulbecco’s Modified Eagle’s Medium (DMEM) containing L-glutamine (Sigma Aldrich) supplemented with 10% FBS (PAA) and 1% antibiotic/antimycotic solution (PAA). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO₂. Culture medium was changed every 2 to 3 days. Samples were collected at Days 1, 4, 8 and 14 timepoints for Picogreen Assay quantification and filament actin staining, and more samples were collected at Days 4, 8 and 14 timepoints for Myosin Heavy Chain (MHC) staining and PCR cDNA synthesis. PBS and 0.05% trypsin-EDTA obtained from Invitrogen were used for washing and cell detachment purposes respectively.

2.6. Quanti-iT picogreen dsDNA assay Kit (Invitrogen, Australia)
A range of solutions with known cell concentration (5000, 10 000, 20 000, 40 000 and 80 000) were mixed in 1:1 ratio with Picogreen working solution prepared according to the vendor’s instructions (fluorescence emission is 520 nm, excitation wavelength is 480 nm) using Infinite 200 microplate reader (Tecan Inc., Maennedorf, Switzerland). A calibration curve was constructed from plotting absorbance wavelengths against the known cell concentration.

Cells on the samples collected from the four timepoints were washed thrice in PBS and then lysed in 0.1 vol. % Triton X-100 solution, supported with gentle pipetting for 30 min, and put through freeze-thawing thrice to ensure that cells were completely lysed. Picogreen working solution was prepared according to the vendor’s instructions. Picogreen working solution was then added at a 1:1 ratio (100 μl:100 μl) per well to the lysed cells in black 96-well plate, to prevent degradation of the light-sensitive picogreen reagent and incubated for at least 5 min at room temperature. A blank well was also generated with a mixture of TE buffer and Triton X-100. As with the calibration steps, fluorescence emission was then measured using Infinite 200 microplate reader at 520 nm under an excitation wavelength of 480 nm. Fluorescence resulting from the blank was subtracted from that of the samples before they were being compared with the above-obtained calibration curve to obtain cell concentration.

2.7. Cytoskeleton (CSK) and MHC imaging
Cell samples at respective timepoints were fixed with 4% paraformaldehyde (PFA) in PBS for 10 min, washed twice with PBS and permeabilized with 0.1% PBS triton-X100 for 15 min. For cytoskeleton imaging, f-actin in cell samples were stained in the dark with tetramethyl rhodamine isothiocyanate-conjugated phalloidin (Chemicon) (1:500) and incubated for 1 h. Cell nuclei were stained with 4′,6-diamidino-2-phenylindole DAPI (Chemicon) (1:300) for 5 min.
For MHC imaging, cells were likewise fixed and permeabilized, then blocked with 5% normal goat serum (Vector Labs) for 1 h at room temperature followed by addition of primary antibodies. The primary antibodies used are as follows: mouse monoclonal anti cardiac MHC (ab15, Abcam) and cardiac Troponin T (1:400, abcam). Alexa Fluor secondary antibodies 488 goat anti mouse IgG, were used. Samples were incubated with the respective secondary antibodies for 1 h at room temperature. Fluorescence images were visualized with a Nikon 80i eclipse (Nikon, Japan) upright microscope and captured with a Nikon DS-Fi1 digital camera (Nikon, Japan) using 20X and 40X objective lens.

2.8. Quantitative real time polymerase chain reaction (qRT-PCR)
qRT-PCR was done as referring to our previous work [20–22]. Specific genes expression in different differentiation directions of myogenic, osteogenic and neurogenic directions, the sequence of gene primers is shown in the tables 1–3 below. Total RNA (tRNA) was extracted with RNeasy mini kit (Qiagen). The concentration and quality of the extracted tRNA was determined spectrophotometrically (Nanodrop-N100, Thermo Scientific). First strand complementary DNA (cDNA) was synthesized with oligo dT and ImProm-II reverse transcription system (Promega) according to the manufacturer’s protocol. qRT-PCR was performed on a CFX96 real time PCR detection system (Bio-Rad Laboratories, Inc., USA). The reaction mix comprises of 10uL of KAPA SYBR FAST master mix (2x) universal, 0.4 μL of forward and reverse primers (10 μM) respectively, 1 μL of diluted cDNA and PCR grade water to make up a final volume of 20 μL. The following cycling thermal profile

### Table 1. Primer sequences of myogenic genes used for the RT-qPCR analysis.

| Gene symbol | Primer sequences (5’-3’) |
|-------------|-------------------------|
| cTnT        | F: TCTCAGAAAGATCAAGACGA R: GCCGCTGACTTTTAGCTT |
| GATA-4      | F: CCAAGCTTCTAGTCAGT GT R: GTGTTTCCAGAGCTGCTG |
| MHC7        | F: CACTGTAAGCTTTTGTAGTG R: TAGGCAGACTTTTGACTGCT |
| MyoD1       | F: CGGGCAAGCTGCTAGGAG R: GCGACTAGAGAGGCAAGTC |
| Nkx2.5      | F: GACCTCGGGCGGATAGGA R: TGGCGGCTGTCGTTAAC |

### Table 2. Primer sequences of osteogenic genes used for the RT-qPCR analysis.

| Gene symbol | Primer sequences (5’-3’) |
|-------------|-------------------------|
| ALPL        | F: CTCCTCAAGAGCTACACACCC R: AATGCGCCACAGATTTCCAGC |
| OCN         | F: GGACACATTCTTCGTCACCTGT R: GTTCACCTACATTGGTCCTG |
| ON          | F: AGACACCGCATTTGAGG GT A R: GGTCACAGGGTCTGGAAAAAGC |
| RUNx-2      | F: TCCTATGACACCAGTCTAACCCCT R: GGCTCTTCTTACTGAGAGTGGA |

### Table 3. Primer sequences of neurogenic genes used for the RT-qPCR analysis.

| Gene symbol | Primer sequences (5’-3’) |
|-------------|-------------------------|
| MAP2        | F: CAGGAATTGACCTCCCTAGACGC R: TCCTTACCCAGCTCTTTGTTCGC |
| Nestin      | F: CACAGCGACGAGGCTCCTC R: CTTCTACGGCTCCTGTCTGAGT |
| NeuroD1     | F: GCCTTGTGATTTCTAAACGCG A R: GTGGGTGGGAGATAAGCCCTT |
| Tublin      | F: GGCAAAGGTCCTCCTACAG R: GCAGTGCACTTTTCACTTC |

For MHC imaging, cells were likewise fixed and permeabilized, then blocked with 5% normal goat serum (Vector Labs) for 1 h at room temperature followed by addition of primary antibodies. The primary antibodies used are as follows: mouse monoclonal anti cardiac MHC (ab15, Abcam) and cardiac Troponin T (1:400, abcam). Alexa Fluor secondary antibodies 488 goat anti mouse IgG, were used. Samples were incubated with the respective secondary antibodies for 1 h at room temperature. Fluorescence images were visualized with a Nikon 80i eclipse (Nikon, Japan) upright microscope and captured with a Nikon DS-Fi1 digital camera (Nikon, Japan) using 20X and 40X objective lens.
was employed: enzyme activation at 95 °C for 3 min, 45 cycles of 95 °C for 3 s and 60 °C for 20 s followed by a dissociation step to analyses the melt curve.

3. Results and discussion

3.1. Fabrication of substrates with varied stiffness
This report mainly studied how combined physical properties of stiffness and topography affect hMSCs cell behavior. Polydimethylsiloxane (PDMS) was selected as the scaffold materials due to its renowned and established biocompatibility, favorable substrate properties, and the ease of manipulating stiffness properties by varying its cross-linking density. With knowledge that Elastic Modulus, $E$, is related to Storage Modulus, $G$, in the equation $E = 2G(1 + \nu)$, where the poisson’s ratio $\nu$ is 0.5 for elastomeric materials, $E$ value was calculated and tabulated in table 4 for the three films. The 50:1 film was found to have Elastic Modulus of 9.63 kPa, which is closest to the desired moderate stiffness of ~10 kPa. From the graph in figure 1, it was also observed that the stiffness property of PDMS remains stable even up to 40% strain rate. Thus, we successfully fabricated the substrates approximating to the elastic moduli of muscle (8 to 17 kPa), which had been demonstrated to direct stem cells, especially hMSCs, to commit to myoblasts [25].

3.2. PDMS film characterization
It has been reported that pre-coating with ECM proteins could improve cell function on the surface of polymeric materials [27]. Graft a homogenous layer of collagen type I would enhance hMSCs adhesion and attachment [28]. Figure 2 revealed no observable difference in functional groups between lasercut and uncut PDMS films, hence ruling out the possibility that chemical integrity was compromised by the heat and abrasion from microchannel grafting during laser machining process. A comparison of the spectrums between smooth PDMS film prior to surface modification and surface modified film illustrated the presence of two extra peaks at 3325 and 1550 cm$^{-1}$ for the surface modified film. They are wavenumbers related to NH stretching and NH bending respectively, both of which could be found in collagen type I [29]. These NH bonding peaks ascertained that collagen type I had been successfully coated on the PDMS films. IR bands of pure PDMS include stretching vibrations of Si-O at 1100–1000 cm$^{-1}$, Si-CH$_3$ at 800 cm$^{-1}$ and 1250 cm$^{-1}$, Si-O at 875 cm$^{-1}$ and CH at 2950 cm$^{-1}$, all of which accurately spell out the main peaks in the spectra in figure 2, thereby confirming that the peaks came from functional groups in PDMS and proving the absence of impurities [30].

3.3. Cell proliferation
hMSCs were seeded and cultured on the surface modified PDMS substrates with and without microchannels (using TCPs as control group), after which they were collected at four different timepoints over a 14 d culture

| Storage modulus, G (kPa) | Young’s modulus, E (kPa) |
|-------------------------|-------------------------|
| 40:1 PDMS Film          | 8.94 ± 0.23             | ~26.82                  |
| 50:1 PDMS Film          | 3.21 ± 0.08             | ~9.63                   |
| 60:1 PDMS Film          | 2.03 ± 0.03             | ~6.09                   |

Table 4. The conversion between storage modulus and elastic modulus.

Figure 1. The rheological test of PDMS films.
period to observe proliferation patterns (figure 3) examined using Picogreen DNA quantification. Figure 3 shows the absolute cell number per scaffold at Days 1, 4, 8 and 14 after seeding at an initial cell seeding density. The control group without laser machining had higher cell number compared with the uncut PDMS and laser-machined PDMS scaffolds. Initially higher cell numbers were found on lasercut scaffold, which may be due to higher surface area was achieved by laser machining as compare to flat samples. Later on, physical constraint caused by microchannels could be possible factor to reduce proliferation rate. Cells tend to avoid adhering perpendicularly across microchannels due to unfavorable stresses that would result from them.

3.4. Cellular organization
Cell nuclei and F-actin filaments were stained with DAPI (blue) and Phalloidin (red) to observe cell morphology and cytoskeleton arrangement (figure 4). It was apparent from the confocal microscope imaging that the lasercut films induced cells to elongate and align their filaments in the direction of the microchannels since Day 1. On the other hand, cells grown on the uncut films spread out in various directions.

By taking into consideration just the lasercut films over the four timepoints, more cells were observed to reside within the microchannels than on the intervals. This observation was especially prominent by Day 14. Cells residing in the microchannels could have been shielded from fluidic pressures caused by changing of media, whereas those protruding on the intervals experience greater impact from such pressures and were more prone to being washed away.
3.5. Gene expression by qRT-PCR test

The topographical features among which line-shaped structures were commonly used to culture cells and many studies have been performed that the topographical features can influence cellular behavior, including cells align, proliferation and differentiation [31, 32]. Figure 5–7 revealed gene expression profiles at various timepoints after hMSCs seeding onto the scaffolds. Myogenic markers cTnT, GATA4, MHC7, MyoD1 and Nkx2.5 (figure 5), osteogenic markers ALPL, OCN, ON and RUNx-2 (figure 6), and neurogenic markers MAP2, Nestin, NeuroD1 and Tublin (figure 7) were used to identify hMSC lineage commitment for cells cultured on control, lasercut PDMS films and uncut PDMS films. Gene expression for the cells on lasercut and uncut PDMS films were normalized against control cells for day-to-day comparisons.

As predicted, myogenic differentiation was significantly higher for lasercut PDMS films as compared with uncut films and TCP's since the first timepoint on Day 4, and only grew in significance by Day 14. With favorable substrate stiffness for myogenic differentiation, an up-regulation of myogenic genes was observed from cells on...
the uncut PDMS films for all Days 4, 8 and 14 timepoints. With microchannels that promote elongation and alignment of cells into the myogenic lineage in addition to favorable stiffness, the up-regulation of myogenic genes was further amplified on lasercut films for all 3 timepoints, especially for MHC7 on Day 8, which existed thrice as much as that on TCPs.

Although the upregulation of myogenic differentiation was still observable on Day 14 for both lasercut and uncut films as compared to TCPs, there was a significant drop in from 3 folds to 2 folds for MHC7 and 2.5 folds
to 1.5 folds for MyoD1 on lasercut films while the remaining markers showed unnotably difference from Day 8 results. This could imply that up-regulation in myogenic differentiation would cease to continue soon beyond the Day 14 timepoint. No observable patterns were found for osteogenic and neurogenic gene expression results.

A back-to-back normalized plot of all osteogenic, neurogenic and myogenic gene expression results on Days 4, 8 and 14 timepoints for the lasercut films further emphasized a significant up-regulation of myogenic differentiation in response to the scaffold properties that were tailored to favor myogenic differentiation, while osteogenic and neurogenic gene expression remained low in significance on Days 4 and 8.

3.6. Immunostaining of MHC

Immunostaining on mature marker of myogenesis, cardiac MHC (green) (figure 8) was conducted to support the differentiation results provided by gene expression analysis. Gene expression analysis and immunostaining of MHC collectively concluded that hMSCs have been successfully directed into myogenic cell differentiation. After 14 d of cell culture, the MHC expression increased gradually with the prolongation of culture time. And it was expressed in the extracellular matrix of whole cell. This result was same with the qPCR of myogenic markers. It was uncovered again on the protein level, the lasercut PDMS with appropriate stiffness has better effect on promoting myogenic differentiation. The topographical properties not only could be tailored to direct hMSCs orientation and differentiation into myogenic lineage, but also further boost of myogenic differentiation was realised by such substrates with additional suitable modulus.

4. Conclusion

The aim of the present work was to investigate femtosecond laser machining microchannels on a surface with moderate stiffness to improve myogenic commitment of hMSCs. The spreading, adhesion, proliferation and differentiation of hMSCs on lasercut and unlasercut films were studied. Lasercut microchannels on the substrates with moderate stiffness induced cell alignment along with the direction of microchannels and stem cells differentiation towards myogenic lineage. Thus, this work exhibited that surface topography together with proper modulus do improved stem cell myogenesis. And we envisage that our technique could enable spatial patterning of cells in a controllable manner, giving rise to advanced capabilities in cell biology research.

Acknowledgments

This work was supported by Key Laboratory of Orthopaedics of Zhejiang Province (Grant No: ZJGK1803Y), National Natural Science Foundation of China (81601626, 51771069), National Key Research and Development Program of China (Project No. 2016YFB1101103) and startup funding from Wenzhou government (WIBEZD2014005-04).
References

[1] Parekkadan B and Milwid J M 2010 Mesenchymal stem cells as therapeutics Annu. Rev. Biomed. Eng. 12 87–117
[2] Buxboim A, Rajagopal K, Brown A E X and Discher D E 2010 How deeply cells feel: methods for thin gels J. Phys.: Condens. Matter 22 194116–25
[3] Han C M, Wang S Y, Lai P P and Cen H H 2007 Human bone marrow-derived mesenchymal stem cells differentiate into epidermal-like cells in vitro Differentiation 75 292–8
[4] Shinya W et al 2012 Modulation of osteogenic differentiation in hMSCs cells by submicron topographically-patterned ridges and grooves Biomaterials 33 128–36
[5] Zheng Y H, Xiong W, Su K, Kuang S J and Zhang Z G 2013 Multilineage differentiation of human bone marrow mesenchymal stem cells in vitro and in vivo Exp. Ther. Med. 5 1576–80
[6] Hutmacher D W 2000 Scaffolds in tissue engineering bone and cartilage Biomaterials 21 2329–43
[7] Lee S H and Shin H 2007 Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering Adv. Drug Deliv. Rev. 59 339–59
[8] Baptista L S et al 2018 Adult stem cells spheroids to optimize cell colonization in scaffolds for cartilage and bone tissue engineering Int. J. Mol. Sci. 19 1285
[9] Lutolf M P and Hubbell J A 2005 Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering Nat. Biotechnol. 23 47–55
[10] Hwang N S, Varghese S and Eliseeff J 2008 Controlled differentiation of stem cells Adv. Drug Deliv. Rev. 60 199–214
[11] Yong X et al 2017 A mesoporous silica composite scaffold: cell behaviors, bio mineralization and mechanical properties Appl. Surf. Sci. 423 314–21
[12] Lund A W, Yener B, Stegemann J P and Plopper G E 2009 The natural and engineered 3D microenvironment as a regulatory cue during stem cell fate determination Tissue Eng. B 15 371–80
[13] Tay C Y, Irvine S A, Boey F Y C, Tan L P and Venkatraman S 2011 Micro-/Nano-engineered cellular responses for soft tissue engineering and biomedical applications Small 7 1361–78
[14] Higuchi A, Ling Q-D, Chang Y, Hsu S-T and Umezawa A 2013 Physical cues of biomaterials guide stem cell differentiation fate Chem. Rev. 113 3297–328
[15] Martínez E et al 2009 Stem cell differentiation by functionalized micro- and nanostructured surfaces Nanomedicine 4 65–82
[16] Wochowski C et al 2005 Femtosecond-laser induced formation of grating structures in planar polymer substrates J. Opt. A: Pure Appl. Opt. 7 493–501
[17] Cunha A et al 2015 Human mesenchymal stem cell behavior on femtosecond laser-textured Ti-6Al-4V surfaces Nanomedicine 10 725–39
[18] Aguilar C A, Lu Y, Mao S and Chen S 2005 Direct micro-patterning of biodegradable polymers using ultraviolet and femtosecond lasers Biomaterials 26 7642–9
[19] Yeong W Y et al 2010 Multiscale topological guidance for cell alignment via direct laser writing on biodegradable polymer Tissue Eng. C 16 1011–21
[20] Li H et al 2012 Direct laser machining-induced topographic pattern promotes up-regulation of myogenic markers in human mesenchymal stem cells Acta Biomater. 8 531–9
[21] Li H et al 2013 Human mesenchymal stem-cell behaviour on direct laser micropatterned electrosprun scaffolds with hierarchical structures Macromol. Biosci. 13 299–310
[22] Li H, Wen F, Wang X and Tan L P 2015 Role of RhoA/Rho kinase signaling pathway in microgroove induced stem cell myogenic differentiation Biointerphases 10 021003
[23] Tay C Y et al 2011 Bio-inspired micropatterned platform to steer stem cell differentiation Small 7 1416–21
[24] Holle A W et al 2013 In situ mechanotransduction via vinculin regulates stem cell differentiation Stem Cells 31 2467–77
[25] Engler A J, Sen S, Sweeney H L and Discher D E 2006 Matrix elasticity directs stem cell lineage specification Cell 126 677
[26] Yu H et al 2013 A Bio-inspired platform to modulate myogenic differentiation of human mesenchymal stem cells through focal adhesion regulation Adv. Healthcare Mater. 2 442–9
[27] Wipff P et al 2009 The covalent attachment of adhesion molecules to silicone membranes for cell stretching applications Biomaterials 30 1781–9
[28] Qian Z, Ross D, Jia W, Xing Q and Zhao F 2018 Bioactive polydimethylsiloxane surface for optimal human mesenchymal stem cell sheet culture Bioactive Mater. 3 167–73
[29] Zhao Y-H and Chi Y-J 2009 Characterization of collagen from eggshell membrane Biotechnology 8 254–8
[30] Petibois C, Gosseilhou G, Wehbe K, Delage J-P and Delérie G 2006 Analysis of type I and IV collagens by FT-IR spectroscopy and imaging for a molecular investigation of skeletal muscle connective tissue Anal. Bioanal. Chem. 386 1961–6
[31] Yim E K, Pang S W and Leong K W 2007 Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage Exp. Cell Res. 313 1820–9
[32] Kulangara K and Leong K W 2009 Substrate topography shapes cell function Soft Matter 5 4072–6