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Mechanical behavior of human mesenchymal stem cells during adipogenic and osteogenic differentiation

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ABSTRACT

Human mesenchymal stem cells (hMSCs) have gained widespread attention in the field of tissue engineering but not much is known about the changes of mechanical properties during the process of cell lineage commitment and the mechanisms of these behaviors. It is believed that exploring the inter-relations between stem cells mechanical properties and lineage commitment will shed light on the mechanobiology aspect of differentiation. hMSCs were cultured in adipogenic and osteogenic mediums and the elastic moduli were monitored using micropipette aspiration. It was found that hMSCs undergoing osteogenesis have an instantaneous Young's modulus of 890 ± 219 Pa and an equilibrium Young's modulus of 224 ± 40 Pa, each is about 2-fold higher than the control group. Interestingly, cells cultured in adipogenic medium exhibited a slight increase in the cellular modulus followed by a decrease relative to that of the control group. Gene expression study was employed to gain insights into this phenomenon. Concomitant up regulation of actin binding filamin A (FLNa) and γ-Tubulin with the cellular elastic modulus indicated their important role in mechanical regulation during hMSCs differentiation. Statistical results showed that cell shape and cell area changed with cellular mechanical properties, which means that cell morphology has a close relation with cell elastic modulus in the initial stage of differentiation. Collectively, these results provide a quantitative description of hMSCs mechanical behavior during the process of differentiation as well as the possible accompanying mechanism at the biomolecular level.

Introduction

It is well known that stem cells are sensitive to their microenvironment, and their responses to the niche are always introduced through the cytoskeleton and accompanied with changes in cell stiffness [1]. On the other hand, the mechanical properties of cells can affect their physical interactions with the surrounding extracellular matrix [2]. Darling et al. studied the viscoelastic behavior of differentiated human mesenchymal stem cells (hMSCs) such as chondrocytes, osteoblasts, and adipocytes using atomic force microscopy (AFM), and found that these cells exhibit distinct mechanical properties, with osteoblasts being stiffer than chondrocytes, and both being stiffer than adipocytes [3]. These interesting observations affirmed that cellular differentiation and mechanical properties are closely related and served as an inspiration for further investigation on how the progress of differentiation could be correlated to the evolution of mechanical properties of stem cells.

Many techniques have been developed to determine the mechanical properties of cells, such as AFM, laser tweezers, magnetic twisting cytometry, and fluid shear flow. However, in these techniques, external factors (e.g., electric fields, osmotic pressure and fluid flow) are inextricably coupled within the cell matrix which will lead to "impure" results which contain external force effects [4]. Besides that, AFM and magnetic twisting cytometry are localized measurements, which are not representative enough for the whole mechanical properties of the cell [5]. In our study, we employed micropipette aspiration since it is a mechanical loading of an entire cell, and the measurement has the advantage of eliminating the cell matrix interaction and other factors mentioned above which then may better represent the mechanical properties of a whole cell. This technique has been widely used to probe the mechanical properties of adherent cells such as hMSCs [6], chondrocytes [7], fibroblasts [8], and endothelial cells [9].

To probe deeper into the cause of changes in cellular mechanical properties, Pablo Rodriguez et al. reported that cytoskeleton changed from a large number of thin actin filament bundles in undifferentiated hMSCs to a few thick actin filament bundles in osteogenic differentiated hMSCs [10]. In the study of cell elastic modulus in osteogenic differentiation, Titushkin et al. found that the elastic modulus changed with reorganization of the cytoskeleton network. They then postulated that the actin filament played

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the essential role in cell mechanical properties [11]. Among these studies, cell elastic modulus was measured and actin filaments were immunostained to show actin development during the differentiation process. However, very limited work has been done at the biomolecular level to explore the correlation between cytoskeleton genes and the change in mechanical properties of the cell. Thus the objectives of this study are to monitor stem cell elastic modulus using micropipette aspiration during the process of differentiation, and to establish the correlation between the change in mechanical properties and cytoskeleton gene expression using quantitative real-time polymerase chain reaction (qRT-PCR). Furthermore, mechanical property change is also linked to cell morphology change, using statistical method.

Materials and methods

hMSCs culture and induced differentiation. Cryopreserved hMSC, derived from human bone marrow, were purchased from Cambrex Inc. (Walkersville). The experiments described herein were performed using passage 5. For induced differentiation, hMSCs were seeded at 2000 cells/cm^2 and were cultured in complete medium made of DMEM low glucose medium (Invitrogen), 16.5% FBS and 1% penicillin/streptomycin (Nagase) at 37 °C in a humidified atmosphere of 5% CO_2. When the cells reached 70% confluence, cell medium was aspirated and appropriated induction media (set this time point as day 0) were introduced. The induced differentiation treatment methods were used as previously described [12]. Briefly, adipogenesis medium was complete medium supplement with a cocktail of 0.5 μM dexamethasone, 0.5 μM isobutylmethylxanthine, and 50 μM indomethacin. Osteogenesis medium was complete medium supplement of a cocktail of 10 nM dexamethasone, 20 mM β-glycerol phosphate, and 50 μM L-ascorbic acid 2-phosphate. All the chemicals for induction medium were purchased from Sigma–Aldrich. The hMSCs in the control group were cultured in complete medium without any induction factors. The histochemical characterization of hMSCs differentiation was shown in supplementary materials.

Micropipette aspiration and viscoelastic model. Experimental procedures and theoretical modeling were based on previous work described in detail by Tan et al. [13]. Briefly, at selected time points, hMSCs were detached with 0.25% trypsin–EDTA for 4 min, pelleted, and resuspended in pre-warm up PBS. Micropipettes were pulled and refined from borosilicate glass capillary tubes (Sutter Instrument, USA) in a Flaming/Brown micropipette puller (P-97, Sutter Instrument, USA) and a microforge (Narishige, Japan), to the desired tip with a inner diameter from 8 to 11 μm. Pressures were applied to the surface of a cell with a in-line pressure transducer (Model DP15–30, Validyne Engineering Corp., USA). Before the start of each aspiration, the equilibrium pressure, where the stem cell was neither aspirated nor pushed away by the micropipette (Fig. 1A), was recorded. Then a constant pressure, ΔP was applied for 300 s and the whole aspiration process was recorded using an inverted microscope (Zeiss Axiovert S100, Germany) with a CCD camera (Diagnostic Instruments Inc., USA) through a 100× objective lens (Fig. 1B and C). The aspiration length of the cell was measured (with an accuracy of ±0.5 μm) using AxioVision software version 4.7 (Zeiss, Germany) at regular time intervals and the length was plotted with respect to time (Fig. 1D). 10 cells were measured in each group and the measurement of each group was completed within 1 h after trypsinization to ensure reliability.

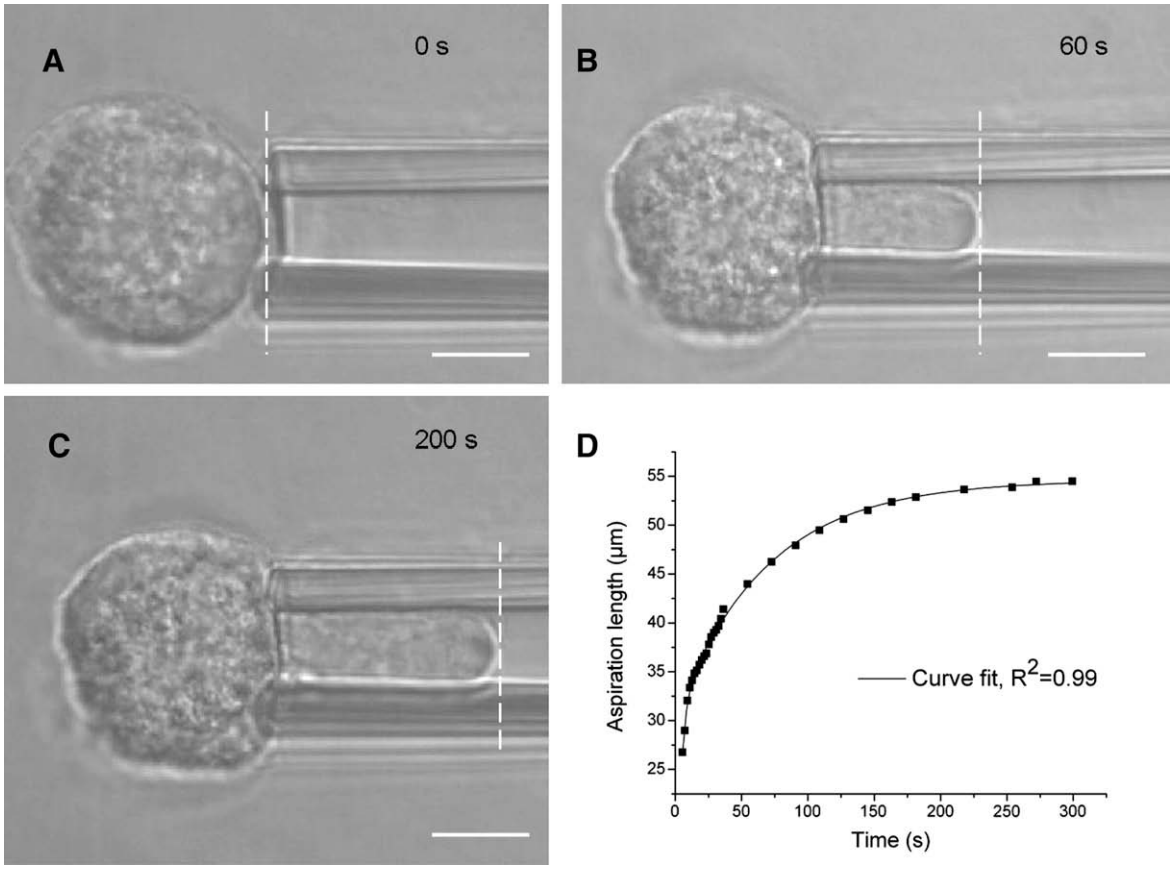

Fig. 1. Characterization of a single hMSC elastic modulus with micropipette aspiration. At t = 0 s (A), the hMSC was closed to the micropipette under the equilibrium pressure, was neither aspirated nor pushed away. At t = 60 and 200 s after step aspirated pressure applied (B,C), the hMSC was aspirated into the micropipette. Typical pattern of aspiration length with respect to time is shown in (D) with a nonlinear regression curve fit using Eq. (1). The dashed line showed the front protrusion of the hMSCs and scale bar showed 10 μm.

For selection of cells, the suspended cells were first visually inspected and cells that were most representative of the overall morphology (in terms of size and shape) are selected for measurement. The aspiration was done in such a way that it was away from the cell nucleus, because cell nuclei are known to be harder than the cytoplasm. Besides that, cells with blebs, which mean that the microtubules were affected, were not selected for measurement.

According to the methods of Tan et al., a linear, three-parameter viscoelastic solid model was used to determine the elastic moduli of hMSCs [13]. The displacement of cell into the micropipette as a function of time, $L(t)$, is:

$$L(t) = \frac{\Phi a \Delta P}{\pi k_1}\left[1 - \frac{k_2}{k_1 + k_2} e^{-t/\tau}\right] \quad (1)$$

where Φ is related to the ratio of the micropipette wall thickness to the pipette radius: in this study, $\Phi = 2.1$ was used based on a punch model [14]. a is inner radius, ΔP is the applied aspiration pressure, τ is the exponential time constant, while k_1 and k_2 are elastic constants which can be determined by solving Eq. (1) using nonlinear regression [13]. The instantaneous Young's modulus, E_0, and the equilibrium Young's modulus, E_∞, are:

$$E_0 = \frac{3}{2}(k_1 + k_2), \quad E_\infty = \frac{3}{2}k_1 \quad (2)$$

Morphological analysis. Cell morphology was analyzed in terms of cell area and cell circularity by ImageJ version 1.42 h (National Institute of Mental Health). One hundred and twenty cells were analyzed in each group. Similar considerations as the micropipette study were taken when selecting cells.

Table 1
Primer sequence and product size for qRT-PCR.

Genbank accession number	Gene target	Primer Sequence (5′–3′)	Product size (bp)
NM_001101	β-Actin	Sense: CATGTACGTTGCTATCCAGGC Antisense: CTCCTTAATGTCACGCACGAT	250
NM_002046	GAPDH	Sense: CATGAGAAGTATGACAACAGCCT Antisense: AGTCCTTCCACGATACCAAAGT	113
NM_001928	Adipsin	Sense: GACACCATCGACCACGACC Antisense: GCCACGTCGCAGAGAGTTC	128
NM_000230	Leptin	Sense: AGATCCTCACCAGTATGCCTT Antisense: CTCTGTGGAGTAGCCTGAAGC	179
NM_004967	BSP	Sense: TGGATGAAAACGAACAAGGCA Antisense: AAACCCACCATTTGGAGAGGT	200
NM_000582	Ocal	Sense: CACTCCTCGCCCTATTGGC Antisense: GCCTGGGTCTCTTCACTACCT	138
NM_001456.3	FLNa	Sense: CCAAGCCTAGCAAGGTGAAG Antisense: TGTCAGCGAAGAGGATGTTG	234
NM_003380	Vimentin	Sense: AGAACTTTGCCGTTGAAGCTG Antisense: CCAGAGGGAGTGAATCCAGATTA	255
NM_001070	γ-Tubulin	Sense: GACGCAGAATGCAGACTGTGT Antisense: CGTAGTGAGAGGGGTGTAGC	250

qRT-PCR. The qRT-PCR was performed according to the methods previous described [15]. Briefly, total RNA was extracted with RNeasy mini kit (Qiagen, Valencia, USA) then was synthesized into cDNA with ImProm-II™ (Promega Corp., MI, USA). qRT-PCR was performed on a CFX96 RT-PCR detection system (Bio-Rad Laboratories, Inc.) with KAPA SYBR FAST master mix universal (Kapa Biosystems, Inc., USA). Primers specific to the targeted genes (Table 1) were obtained from primer bank [16]. Relative quantification of gene expression was analyzed with the CFX manager software (Bio-Rad Laboratories, Inc.), and the Relative Expression Software Tool 2008 (REST 2008). GAPDH and β-actin were used as endogenous housekeeping genes.

Statistical analysis. All assays were repeated three times for each time point and were expressed as means ± standard deviation (SD). Statistical analysis used SigmaPlot version 9.0 (SYSTAT Software, Inc., USA). Student's t-test assuming unequal variance was used for determining statistically differences. A value of $p < 0.05$ was considered statistically significant.

Results

Mechanical properties changes in hMSCs during adipogenic and osteogenic differentiation

In the aspiration experiment, all of the hMSCs and induced differentiated cells exhibited a typical monotonic viscoelastic behavior, and possessed a high mean correlation coefficient ($R^2 \geqslant 0.98$) using nonlinear regression when fitted in a three-parameter viscoelastic solid model [13] (Fig. 1D). E_0 is assumed to be a better indicator of the instantaneous response of a cell which is independent of time [17]. E_∞ is obtained at adequately long time to represent equilibrium response of cell elasticity. Both of them are representative parameters for cell mechanical properties.

From calculations, it was observed that both the E_0 (Fig. 2A) and E_∞ (Fig. 2B) of the control group did not show any obvious change during the entire experiment (from 457 ± 88 Pa to 466 ± 87 Pa, and 98 ± 37 Pa to 116 ± 15 Pa, respectively). These elastic moduli fall in between those of soft adipogenic and stiff osteogenic cells and the data are in good agreement with previous report [13]. While in the adipogenic induced experimental group, E_0 and E_∞ peaked at day 7 of induction (671 ± 158 Pa and 102 ± 39 Pa, respectively) and reduced thereafter to 420 ± 52 Pa and 87 ± 23 Pa, respectively. The final elastic moduli at day 21 were lower than those of the control group ($p < 0.05$). For the hMSCs cultured in osteogenic medium, the Young's modulus increased with time throughout the experiment. By the end of the experiment, E_0 and E_∞ of the cells cultured in osteogenic induction medium increased from 457 ± 88 Pa and 98 ± 37 Pa to 890 ± 219 Pa and 224 ± 40 Pa, respectively, which are nearly two times higher than those of the control group ($p < 0.01$).

Cytoskeleton genes analysis

Representative genes of three major classes of structural proteins were selected to investigate cytoskeleton rearrangement which related with the cell elastic modulus at the transcript level. Actin binding filamin A (FLNa) encodes the actin binding protein which cross-links actin filaments and links actin filaments to membrane glycoproteins [18]. γ-Tubulin encodes a number of tubulin superfamily, which localizes to the centrosome where it binds to microtubules as part of a complex referred to as the γ-Tubulin ring complex [19]. It mediates microtubule nucleation and is required for microtubule formation [20]. Vimentin encodes intermediate filament family proteins such as vimentin and desmin, which along with actin filament and microtubules make up the cytoskeleton

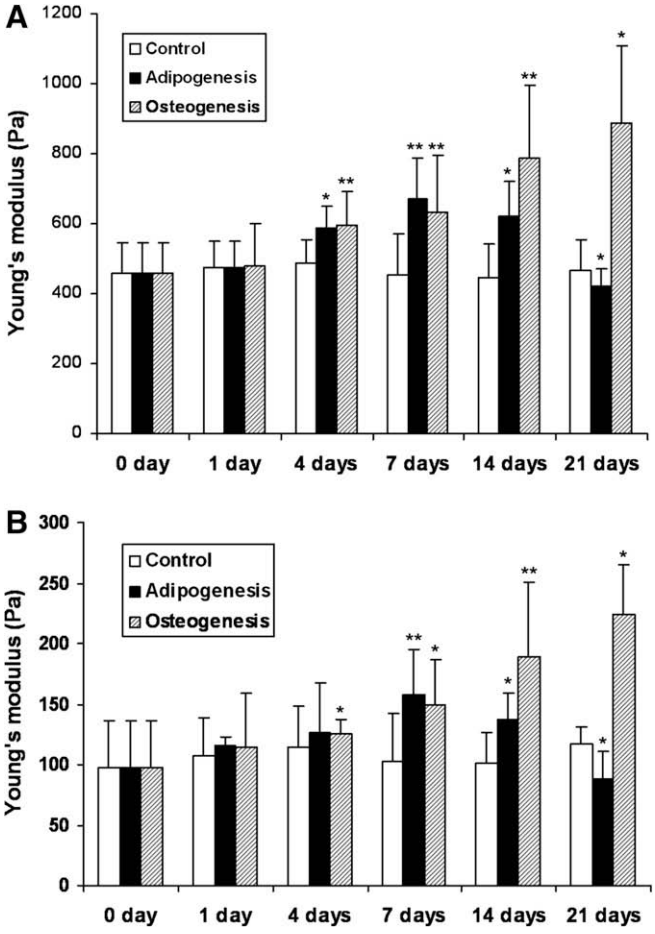

Fig. 2. hMSCs mechanical properties during adipogenic and osteogenic differentiation. E_0 is shown in (A), and E_∞ is shown in (B). $^{*}p < 0.05$ and $^{**}p < 0.01$ as compared with control group.

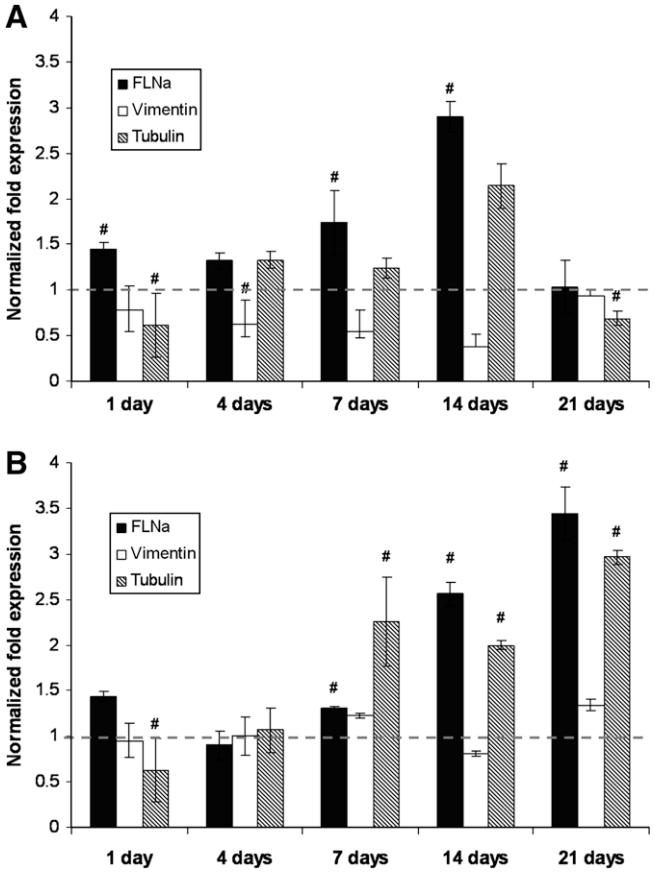

Fig. 3. Relative cytoskeleton genes level expression analysis of adipogenesis (A) and osteogenesis (B). Relative gene levels (i.e., $\Delta\Delta C_T$) were normalized to the control group indicated by the dashed line. # showed significant difference as compared with control group analyzed with REST 2008.

[21], and it functions as an organizer of a number of critical proteins involved in attachment, migration, and cell signaling [22].

It is shown, from the results of cytoskeleton genes expression of the cells cultured in adipogenic medium (Fig. 3A) that both the FLNa and γ-Tubulin were up regulated during the initial stage of differentiation (during the first 2 weeks) before decreasing to a level close (FLNa) or lower (γ-Tubulin) to the control group at 21 days of culture. The trend of these two cytoskeleton gene expression matched well with that of the viscoelastic properties shown in Fig. 2A. For the intermediate filament code gene vimentin, it is seen that the gene was down regulated during the first week in induction medium, and later returned to the same level as compared with the control group, but did not show significant difference.

It is shown in Fig. 3B from the cytoskeleton genes expression of the cells cultured in osteogenic medium that both the FLNa and γ-Tubulin genes exhibit significantly up regulation trend during differentiation process. FLNa increased to as much as 3.5-fold while γ-Tubulin increased to nearly 3-fold of the control group at the end of the experiment. This growing trend matched that of the growing cell elastic modulus shown in Fig. 2B. For the intermediate filament code gene vimentin, no significant differences were seen during the differentiation process as compared with the control group.

Cell morphological analysis

Cell shape and cell spreading behavior have a close relationship with stem cell differentiation and their mechanical properties [23,24]. In light of this, cell spreading area and circularity during the hMSCs differentiation were studied and analyzed using statistical method, in an attempt to correlate to change in cell elastic modulus.

The statistical results of cell spreading area of hMSC are shown Fig. 4A. The area of cells cultured in adipogenic medium increased to a maximum about 1.2×10^4 μm^2, 63% higher than the control group, on the 7th day, and it then decreased to 4×10^3 μm^2, 75% of the control group on day 21 of culture. For the cells cultured in osteogenic medium, the area of the cell also increased initially, followed by a decrease due to the confluence. Moreover, the average area of cells in osteogenic medium was significantly larger than cells both in the basal and in the adipogenic medium ($p < 0.01$) groups at the end of the experiment.

The circularity results of hMSCs during differentiation are shown Fig. 4B. The circularity of the cells cultured in adipogenic medium increased with time, reaching a value of 0.72 ± 0.18, indicative of a rounded shape. The circularity of cells cultured in osteogenic medium increased with time as well in the first week, but they remained low at 0.39 ± 0.03 by the end of the experiment, suggesting that the cells adopted a polygonal shape. As for the control group, the cell shape did not show significant difference throughout the experiment where it remained in elongated/spindle shape.

Discussion

Over the past decades, various hypotheses have been put forward to emphasize the relationship between cytoskeleton

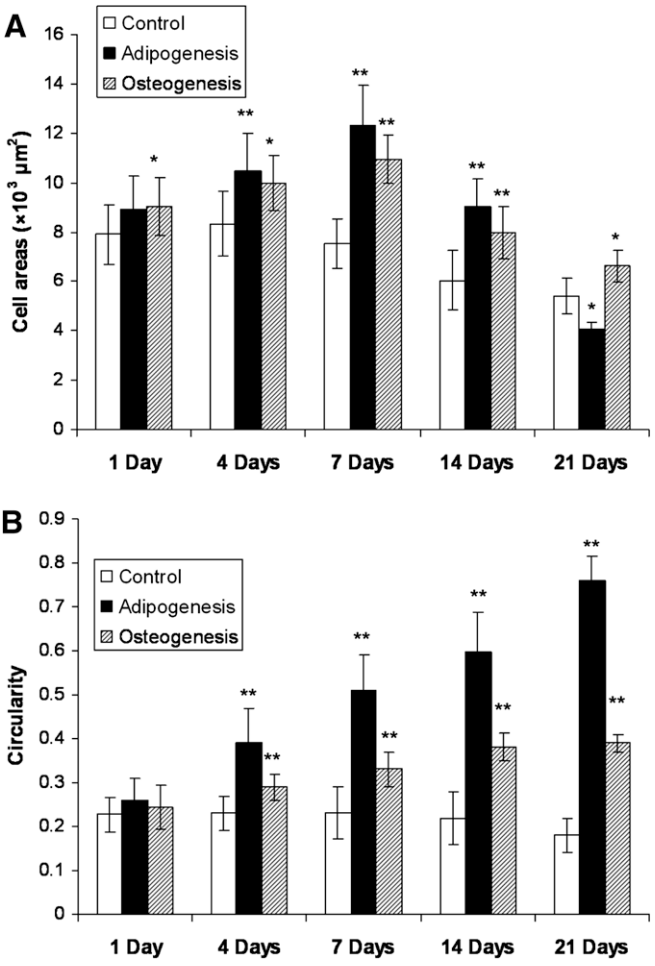

Fig. 4. Cell morphological analysis during hMSCs adipogenic and osteogenic differentiation. Cell areas result is shown in (A) and cell circularity result is shown in (B). $^{*}p < 0.05$ and $^{**}p < 0.01$ when compared with control group.

development with cell elastic modulus, and cellular differentiation, but few studies to date focus on the change in stem cells mechanical properties during the differentiation process and very limited work has been conducted on exploring the mechanism of such changes at the biomolecular level. At present, it is not well understood whether the rearrangements in cytoskeleton organization that take place in hMSCs differentiation are a prerequisite to the cell shape changes and/or for the expression of characteristic biochemical markers of hMSCs differentiation. In our study, we have examined the internal structural changes in hMSCs (reflected by changes in mechanical properties) when they differentiated *in vitro* and have correlated these changes with cytoskeleton development as well as cell morphology. To the best of our knowledge, this is the first study to examine how mechanical properties of hMSCs at different stages of differentiation into adipogenic and osteogenic lineages correlate to cytoskeleton gene regulation.

An important consideration in this experiment is whether trypsinization will affect the mechanical properties of the cells since micropipette aspiration requires adherent cells to be detached for measurement. This effect of trypsin has been reported and was found that the trypsin incubation time is crucial in affecting the microtubules within the cell. It was observed that with 10 min incubation in trypsin, actin system would be affected and microtubules at the edge of the cells would be disrupted and form blebs. But due to the thickness of the cell, microtubules inside the cells still remained intact instead of disintegrating [25]. As additional precaution, in our studies, the trypsinization time was controlled to be within 4 min and cells with blebs were not selected for measurements. The integrity of the cells was confirmed by GFP-tubulin expression showing that the microtubules have not been damaged after trypsinization (Fig. S3). Besides that, the viscoelastic behavior was found to fit well into the established solid model for calculating cell modulus.

Histochemical staining showed in Figs. S1 and S2 showed that on day 21, most of the hMSCs were differentiated. The cells that did not show positive staining on day 21 were stained positive by day 26, showing that the cells selected in mechanical measurement were undergoing differentiation during the mechanical test.

We postulate that the change in mechanical properties in hMSCs undergoing adipogenesis proceeds in two stages. In the first stage, factors in the induction medium induced rearrangement of the cytoskeletal system resulting in a higher cell spreading area, and accompanied by stiffening of the cells. This process is also associated with an up regulation of FLNa and γ-Tubulin gene expression, as evident in the gene analysis results. Reports show that FLNa coded filament A can increase cell stiffness by 200–400% [26], while microtubules were essential for cell structure change during adipogenesis, and bear mechanical loads that are transmitted in cytoplasm [27]. Taken together, cell spreading and the actin filaments as well as tubulin network have a tight relation with the initial change in mechanical properties of cells in adipogenic medium. In the second stage, after 7 days in induction medium, the differentiating cells stopped spreading, and appreciable differentiation set in as shown by lipid staining. At the same time, the polymerization and cross-linking level of actin had decreased, reflected by the down regulation of FLNa and γ-Tubulin expressions resulting in a decrease in cell elastic modulus. Vimentin expression was down regulated at first, which require the intermediate filaments to dissemble during the differentiation process to a rounder shape [28]. Later the expression of vimentin returned to normal level once the cell shape was stabilized. This process is accompanied by a circularity of more than 0.75 for the cells in adipogenic medium, representing a rounded shape. Taking these observations in tandem, there is a dynamic interplay between both cytoskeletal networks and the cell morphology, and they appear to have a regular role in hMSCs mechanical properties.

In the osteogenic medium, the elastic moduli increased throughout the experiment. According to the gene expression results shown in Fig. 3B, just like the group in adipogenic medium, it can be deduced that actin filaments and tubulin mainly contributed to the changes in the mechanical property of cells in osteogenic medium. After 7 days in the induction medium, the mechanical properties of the cells in osteogenic medium were different from the cells cultured in adipogenic medium in elastic modulus and cytoskeleton development. The elastic moduli of the cells in osteogenic medium continued to increase, and actin polymerization and cytoskeleton cross-linking level were further up regulated. The up regulated expression of FLNa and γ-Tubulin would result in actin filament from thin bundles in undifferentiated hMSCs to thick actin bundles, and well developed actin cytoskeleton and microtubules could promote osteogenesis and suppress adipogenesis of hMSCs [29]. However, vimentin expression did not change much during the osteogenesis process, which could be related by the fact that there was no drastic cell shape change involved. The cell shape evolved gradually into polygonal shape, in which the role of vimentin was not essential.

In this study, the systematic characterization of hMSCs mechanical properties during adipogenic and osteogenic differentiation carried out shed some light on the understanding of the interaction between cellular elastic behavior and stem cell differentiation. The technique used, micropipette aspiration is a reliable technique that

provided results that fit well to established theoretical models for adherent cells.

Conclusions

In summary, we presented a systematic characterization of hMSCs mechanical properties during adipogenic and osteogenic differentiation which will lead to a better understanding of the interaction between cellular elastic behavior and stem cell differentiation, and being able to control stem cell differentiation has great implications in the field of stem cell based tissue engineering and regenerative medicine such as optimizing scaffolds properties for mechanical induced stem cell lineage commitment in tissue engineering, and modulation of the cellular mechanics during hMSCs differentiation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.01.107.

References

[1] D.E. Ingber, Mechanobiology and diseases of mechanotransduction, Annals of Medicine 35 (2003) 564–577.
[2] D.E. Ingber, Cellular mechanotransduction: putting all the pieces together again, The FASEB Journal 20 (2006) 811–827.
[3] E.M. Darling, M. Topel, S. Zauscher, T.P. Vail, F. Guilak, Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes, Journal of Biomechanics 41 (2008) 454–464.
[4] W.R. Jones, H.P. Ting-Beall, G.M. Lee, S.S. Kelley, R.M. Hochmuth, F. Guilak, Alterations in the Young's modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage, Journal of Biomechanics 32 (1999) 119–128.
[5] G. Bao, S. Suresh, Cell and molecular mechanics of biological materials, Nature Materials 2 (2003) 715–725.
[6] P. Wenxiao, E. Petersen, C. Ning, M. Gang, L. Jian Run. F. Zhiqin, L. Kin, K.W. Leong, Viscoelastic properties of human mesenchymal stem cells, in: 27th Annual International Conference of the Engineering in Medicine and Biology Society, 2005 IEEE-EMBS 2005, pp. 4854-4857.
[7] W.R. Trickey, F.P.T. Baaijens, T.A. Laursen, L.G. Alexopoulos, F. Guilak, Determination of the Poisson's ratio of the cell: recovery properties of chondrocytes after release from complete micropipette aspiration, Journal of Biomechanics 39 (2006) 78–87.
[8] O. Thoumine, O. Cardoso, J.J. Meister, Changes in the mechanical properties of fibroblasts during spreading: a micromanipulation study, European Biophysics Journal 28 (1999) 222–234.
[9] M. Sato, N. Ohshima, R.M. Nerem, Viscoelastic properties of cultured porcine aortic endothelial cells exposed to shear stress, Journal of Biomechanics 29 (1996) 461–467.
[10] M.G.S.R.V.C.J. Pablo Rodriguez, Cytoskeletal organization of human mesenchymal stem cells (MSC) changes during their osteogenic differentiation, Journal of Cellular Biochemistry 93 (2004) 721–731.
[11] I. Titushkin, M. Cho, Modulation of cellular mechanics during osteogenic differentiation of human mesenchymal stem cells, Biophysical Journal 93 (2007) 3693–3702.
[12] R.L. Reger, A.H. Tucker, M.R. Wolfe, Differentiation and Characterization of Human MSCs, Mesenchymal Stem Cells, Humana Press, 2008, pp. 93–107.
[13] S. Tan, W. Pan, G. Ma, N. Cai, K. Leong, K. Liao, Viscoelastic behaviour of human mesenchymal stem cells, BMC Cell Biology 9 (2008) 40.
[14] D.P. Theret, M.J. Levesque, M. Sato, R.M. Nerem, L.T. Wheeler, The application of a homogeneous half-space model in the analysis of endothelial-cell micropipette measurements, Journal Biomechanical Engineering 110 (1988) 190–199.
[15] C.Y. Tay, H. Gu, W.S. Leong, H. Yu, H.Q. Li, B.C. Heng, H. Tantang, S.C.J. Loo, L.J. Li, L.P. Tan, Cellular behavior of human mesenchymal stem cells cultured on single-walled carbon nanotube film. Carbon 48 (2010) 1095–1104.
[16] X. Wang, B. Seed, A PCR primer bank for quantitative gene expression analysis, Nucleic Acids Research 31 (2003) e154.
[17] E.M. Darling, S. Zauscher, J.A. Block, F. Guilak, A thin-layer model for viscoelastic, stress-relaxation testing of cells using atomic force microscopy: do cell properties reflect metastatic potential?, Biophysical Journal 92 (2007) 1784–1791
[18] M. Glogauer, P. Arora, D. Chou, P.A. Janmey, G.P. Downey, C.A.G. McCulloch, The role of actin-binding protein 280 in integrin-dependent mechanoprotection, Journal of Biological Chemistry 273 (1998) 1689–1698.
[19] Y. Zheng, M.K. Jung, B.R. Oakley, Gamma-tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome, Cell 65 (1991) 817–823.
[20] Q. Li, H.C. Joshi, Gamma-tubulin is a minus end-specific microtubule binding protein, Journal of Cell Biology 131 (1995) 207–214.
[21] E. Fuchs, K. Weber, Intermediate filaments: structure, dynamics, function and disease, Annual Review of Biochemistry 63 (1994) 345–382.
[22] J. Ivaska, H.M. Pallari, J. Nevo, J.E. Eriksson, Novel functions of vimentin in cell adhesion, migration, and signaling, Experimental Cell Research 313 (2007) 2050–2062.
[23] R. McBeath, D.M. Pirone, C.M. Nelson, K. Bhadriraju, C.S. Chen, Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, Developmental Cell 6 (2004) 483–495.
[24] O. Thoumine, O. Cardoso, J.J. Meister, Changes in the mechanical properties of fibroblasts during spreading: a micromanipulation study, European Biophysics Journal with Biophysics Letters 28 (1999) 222–234.
[25] R.A. Badley, A. Woods, L. Carruthers, D.A. Rees, Cytoskeleton changes in fibroblast adhesion and detachment, Journal of Cell Science 43 (1980) 379–390.
[26] R. Tandon, I. Levental, C. Huang, F.J. Byfield, J. Ziembicki, J.R. Schelling, L.A. Bruggeman, J.R. Sedor, P.A. Janmey, R.T. Miller, HIV infection changes glomerular podocyte cytoskeletal composition and results in distinct cellular mechanical properties, American Journal of Physiology – Renal Physiology 292 (2007) F701–F710.
[27] N. Wang, K. Naruse, D. Stamenović, J.J. Fredberg, S.M. Mijailovich, I.M. Tolić-Nørrelykke, T. Polte, R. Mannix, D.E. Ingber, Mechanical behavior in living cells consistent with the tensegrity model, Proceedings of the National Academy of Sciences of the United States of America 98 (2001) 7765–7770.
[28] R.D. Goldman, S. Khuon, Y.H. Chou, P. Opal, P.M. Steinert, The function of intermediate filaments in cell shape and cytoskeletal integrity, Journal of Cell Biology 134 (1996) 971–983.
[29] V.E. Meyers, M. Zayzafoon, J.T. Douglas, J.M. McDonald, RhoA and cytoskeletal disruption mediate reduced osteoblastogenesis and enhanced adipogenesis of human mesenchymal stem cells in modeled microgravity, Journal of Bone and Mineral Research 20 (2005) 1858–1866.