Adipocyte 2:2, 87–91; April/May/June 2013; © 2013 Landes Bioscience

Adipose-derived stem cell fate is predicted by cellular mechanical properties

Rafael D. González-Cruz[1,2] and Eric M. Darling[1,2,3,4,*]

[1]Department of Molecular Pharmacology, Physiology, and Biotechnology; Brown University; Providence, RI USA; [2]Center for Biomedical Engineering; Brown University; Providence, RI USA; [3]Department of Orthopaedics; Brown University; Providence, RI USA; [4]School of Engineering; Brown University; Providence, RI USA

Keywords: mesenchymal stem cell, fat, cell mechanics, regenerative medicine, atomic force microscopy, differentiation, stem cell enrichment, passaging, cell sorting

Abbreviations: ASC, adipose derived stem cell; MSC, mesenchymal stem cell; AFM, atomic force microscopy; $E_{elastic}$, elastic modulus; E_{equil}, equilibrium modulus; E_0, instantaneous modulus; E_R, relaxed modulus; μ_{app}, apparent viscosity

Adipose-derived stem cells (ASCs) show great promise for tissue engineering applications and cell-based therapies because of their multipotency, relative abundance and immunosuppressive properties. However, ASCs must be isolated from heterogeneous cell populations present in adipose tissue. In this brief report, we provide a concise summary of the history and use of cellular mechanical properties as novel, label-free biomarkers to predict the differentiation potential of ASCs toward adipogenic, osteogenic and chondrogenic lineages. Additionally, we have found that passage number influences the mechanical properties of ASCs along with a discussion of potential environmental factors that could affect these properties. Altogether, this report provides evidence for the reliability of cellular mechanical properties as biomarkers for ASC differentiation potential and outlines how they can be used to sort ASCs with lineage-specific preferences for particular applications.

Introduction

Human, white adipose tissue harbors a population of mesenchymal stem cells (MSCs) known as adipose-derived stem cells (ASCs).[1] These stem cells can differentiate into cells of mesodermal origin, including adipocytes, osteoblasts, chondrocytes, and endothelial cells, among others.[1,2] However, ASCs are highly plastic and their differentiation potential is not limited only to mesodermal tissues (for a review on ASC plasticity, see ref. 3). ASCs have also been shown to differentiate into cells of ectodermal origin, such as neurons and epithelial cells, and cells of endodermal origin, such as hepatocytes, making them an extremely versatile cell source for regenerative therapies.[3]

ASCs are unique because they are more abundant and easier to isolate when compared with other MSC populations in the human body.[4] Additionally, ASCs exhibit immunosuppressive properties, as shown by their ability to inhibit T cell activation responsible for graft vs. host disease in animals and humans.[5,6] Because of the desirable features these cells possess, ASCs are an attractive source for cell-based regenerative therapies and tissue engineering of fat, bone, and cartilage tissues (for reviews of tissue engineering applications using ASCs, see refs. 7–9).[7-9] ASC-based therapies have already been conducted in preclinical animal studies and clinical human trials.[10]

ASC Isolation: A Sorting Problem

ASCs must first be isolated from adipose tissue before being used for cell-based studies. However, lipoaspirate contains other cell types besides ASCs that are often undesirable for regenerative therapies.[11] These cells include fibroblasts, endothelial cells, pericytes and smooth muscle cells. Since they are fully differentiated cell types, their presence can diminish the overall response of the population for tissue-specific applications.[12] Stem cell biologists and engineers are addressing this barrier by exploring different methods to isolate ASCs from heterogeneous populations.

The gold standard for sorting and collecting cells is fluorescence activated cell sorting (FACS). This method consists of identifying cell types based on the expression of surface markers and has been used extensively to isolate many cell types, including ASCs.[11,13-15] Unfortunately, ASCs share several surface receptors with other cells present in adipose tissue.[16,17] Therefore, FACS-based strategies require the use of multiple surface marker labeling schemes to isolate ASCs. Increasing the number of antibody labels used for sorting enhances the specificity of the sorting process itself but also results in a very low cell yield of purified ASCs. As a result, such an approach drastically reduces the total number of cells available for research and clinical applications.

*Correspondence to: Eric M. Darling; Email: Eric_Darling@brown.edu
Submitted: 10/01/12; Revised: 11/26/12; Accepted: 11/27/12
http://dx.doi.org/10.4161/adip.23015

This sorting problem reaches a new level of complexity when considering the expression of these markers as a function of monolayer expansion, as designated by passage number.[18] Specifically, ASCs sorted directly after their isolation from lipoaspirate have different surface receptor profiles than ASCs expanded in culture. As we will discuss later in this report, recent studies suggest that passage number is an important factor affecting ASC differentiation potential. This is problematic because cell-based therapies often require multiple passages to obtain sufficient cell numbers. Altogether, FACS-based sorting strategies are limited by their low cell yield, complex surface receptor labeling schemes required for sorting and passage-dependent surface marker composition.

Cellular Mechanical Properties: A Reference Point

Cellular mechanical properties have emerged as biomarkers for determining cellular phenotype in several contexts, including cancer cell characterization and stem cell differentiation.[19,20] By mechanical properties, we refer to the cell's intrinsic resistance to deformation (elasticity) and flow (viscosity). A material that exhibits elastic properties can fully recover its shape after being deformed by an applied stress. Parameters that help describe a material's elasticity include the elastic modulus ($E_{elastic}$) and equilibrium modulus (E_{equil}). $E_{elastic}$ provides information about the compliance of a material immediately following the application of a stress while E_{equil} provides information about the compliance of a material once the deformation response has reached equilibrium. Materials with high $E_{elastic}$ and E_{equil} values are considered non-compliant or stiff whereas materials with low $E_{elastic}$ and E_{equil} values are considered compliant or soft. On the other hand, materials that are viscous will flow when a stress is applied. Viscous materials can be described by a property termed apparent viscosity (μ_{app}). Materials with high μ_{app} exhibit a greater resistance to flow than materials with low μ_{app} when a stress is applied.

Cells have both solid and liquid components that are responsible for their elastic and viscous behaviors. Because of this dual mechanical behavior, cells are considered viscoelastic materials. Viscoelasticity can be assessed using time- or frequency-dependent tests, and from these, additional parameters can be recorded: instantaneous modulus (E_0) and relaxed modulus (E_R). Materials with large E_0 and E_R values are considered non-compliant whereas those with low E_0 and E_R values are considered compliant. One key difference between elasticity and viscoelasticity is that viscoelastic materials do not fully recover their shape after a stress is applied. Therefore, the viscoelastic properties are time-dependent. E_0 represents the modulus at time t = 0 and depends on the rate of the applied stress while E_R defines the relaxed modulus measured when the material reaches equilibrium. As would be expected, E_0 and E_R often have similar values to $E_{elastic}$ and E_{equil} even though they are extracted from a viscoelastic response curve. We have used these five mechanical properties, along with cell height, as a set of mechanical biomarkers to characterize a variety of cell types, including ASCs.[20-22]

Cellular Mechanical Properties Emerge as ASC Lineage-Specific Biomarkers

Among the elastic and viscous components that comprise the cell, the actin cytoskeleton, which is responsible for the cells' morphology, is also the primary contributor to cellular mechanical properties.[23] In the context of ASC differentiation, previous studies indicate that undifferentiated ASCs have a different cytoskeletal organization than ASCs differentiated toward adipogenic or osteogenic lineages.[24,25] If the organization of the cytoskeleton is changed, either by altering the geometry of the substrate or adding actin polymerization inhibitors to differentiation media, stem cell differentiation also changes.[26,27] The role of the cytoskeleton in determining these mechanical properties has led researchers, including us, to investigate whether these mechanical properties could serve as biomarkers for lineage-specific phenotype. For purposes of this report, we will only mention studies that used atomic force microscopy (AFM)-based microindentation to explore this hypothesis (for a review of this technique, see ref. 28).[28]

In two AFM studies, it was found that the mechanical properties of undifferentiated bone marrow MSCs changed during differentiation while undergoing cytoskeletal rearrangements.[25,29] Specifically, undifferentiated MSCs had lower $E_{elastic}$ values than MSC-derived osteoblasts. The studies' findings provided evidence that changes in cellular mechanical properties were tied to phenotypic changes, most likely as a direct consequence of the differentiation process. What was not investigated was whether the initial mechanical properties of individual MSCs could influence the differentiation response of the cell. In other words, do cellular mechanical biomarkers exist and can they be used to predict stem cell lineage preference?

Part of the answer to these questions came from another study that compared the mechanical properties of spherical and spread ASCs with those of differentiated adipocytes, osteoblasts and chondrocytes via AFM.[20] Results showed clear differences in the mechanical properties among cell types. Specifically, adipocytes were the most compliant, followed by chondrocytes. Osteoblasts were the least compliant cell type. Interestingly, ASCs and MSCs had similar mechanical properties and their compliance was between that of chondrocytes and osteoblasts. Chondrocytes were the most viscous cell type while adipocytes had the largest cell height. The results of this study suggested that the mechanical properties of ASCs could be used as biomarkers to distinguish ASCs from differentiated cells.

Based on the collective findings from these reports, we hypothesized that ASCs in adipose tissue had mechanical properties that were indicative of their differentiation potential toward specific lineages.[21] Because adipose tissue is heterogeneous, we established a set of 32 ASC clonal populations derived from single cells. After five passages, the mechanical properties of a subset of cells from each clonal population were tested using AFM. We acquired mechanical biomarkers for both spherical and spread cell morphologies. Interestingly, significant differences in properties were only found for cells in the rounded

geometry, most likely because that effectively removed cell shape as a contributing factor. Another three clonal population subsets were subjected to adipogenic, osteogenic and chondrogenic differentiation. We assessed the differentiation of ASCs toward these lineages by quantifying lipid accumulation, calcium deposition and sulfated glycosaminoglycan (GAG) production using Oil Red O, Alizarin Red S and dimethylmethylene blue assays, respectively. By performing correlation analyses, we found that the mechanical properties of spherical ASCs correlated with lineage-specific metabolite profiles. Specifically, we found that ASC clones that produced larger amounts of lipids (adipocytic) exhibited greater cell heights, clones that produced more calcified matrix (osteoblastic) exhibited greater $E_{elastic}$ and E_R values and clones that produced more sulfated GAGs (chondrocytic) exhibited greater apparent viscosity values. The results of these experiments supported our hypothesis and showed that differences in the mechanical properties of undifferentiated ASCs could be used to predict their differentiation, and even matrix synthesis potential (**Fig. 1**).

The ASC clones included in the above study were not simply progenitor populations, showing multipotent differentiation capabilities along the adipogenic, osteogenic and chondrogenic lineages. However, the level of metabolite production varied widely among the clones, as did their mechanical properties, emphasizing the heterogeneity of the original ASC population. At this point, we asked whether sorting cells by mechanical biomarkers could produce populations with greater regenerative capacity. To investigate this possibility, we conducted simulations that inspected the mechanical properties of the top 25% of ASC clones for adipogenic, osteogenic and chondrogenic lineages. Indeed, we found that "high potential" ASC clones had similar mechanical properties within each lineage. For example, ASCs exhibiting robust osteogenesis had E_R values above 200 Pa while ASCs exhibiting robust chondrogenesis had μ_{app} values above 1.6 kPa·s. Theoretically, sorting ASC populations for cells exhibiting these characteristics would result in a much more robust, lineage-specific response. The results of these experiments supported our hypothesis and showed that cellular mechanical properties can be used as biomarkers to identify ASCs exhibiting lineage-specific preferences. While certainly an exciting finding, it should be kept in mind that the correlations between mechanical biomarkers and differentiation response were not always strong. Many other factors influence stem cell behavior, with environmental conditions (e.g., biochemical, mechanical and topographical stimuli) being a primary contributor.

Phenotypic Changes of ASCs as a Function of Passage Number: Does It Affect the Mechanical Properties Too?

Despite the abundance of MSCs in adipose tissue when compared with other tissues of mesodermal origin, current isolation techniques such as FACS still yield very low cell numbers. As a result, purified ASC populations must be expanded in vitro until they reach a population size that is feasible for clinical applications. However, previous studies suggest that the time

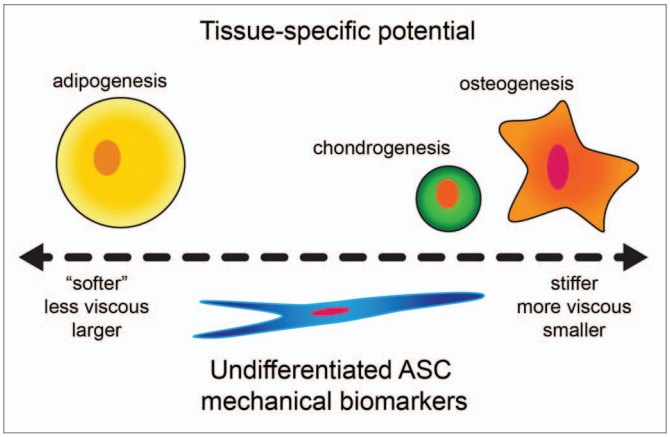

Figure 1. Cellular mechanical properties can predict the differentiation potential of ASCs. Undifferentiated stem cells that are large and compliant tend to be more adipogenic, while undifferentiated cells that are less compliant and more viscous exhibit more robust differentiation along osteogenic and chondrogenic lineages.

cells spend in culture could affect their phenotype and, subsequently, their differentiation potential.[18,30] In one study that used FACS to sort ASCs, cell surface marker expression changed as a function of passage number.[18] Immediately after being isolated, ASCs exhibited high expression levels of CD34, a stem cell-associated marker. However, CD34 expression decreased drastically after only one passage. Similarly, expression levels of hematopoietic marker CD45, initially low in freshly isolated cells, further decreased and became negligible soon after ASCs were expanded.[15] On the other hand, the expression of surface markers such as CD49d, CD73 and CD90, which are expressed at moderate levels in freshly isolated cells, increased dramatically as ASCs were expanded in vitro.[11,18] Altogether, these studies suggest that current ASC expansion conditions effectively enrich for multipotent stem cells of mesenchymal origin.

Additionally, other studies reported that the expression of other surface markers like CD44 and CD73, which are highly expressed in chondrocytes and osteoblasts, increased dramatically in expanded ASCs. These results suggest that ASC surface marker expression resembled that of chondrocytes and osteoblasts as their passage number increased. Separate studies, focusing on changes in ASC differentiation potential as a function of passage number, also reported striking differences between the adipogenic, chondrogenic and osteogenic potential of ASCs at different passages.[30-32] Specifically, results indicated that ASC adipogenic potential was enhanced at early passages (P2–P5) but decreased at later passages. Conversely, ASC chondrogenic and osteogenic potential increased at later passages. Altogether, these results strongly indicate that the time ASCs spend in culture during their expansion strongly influences their fate.

Our previous work investigated the mechanical properties and differentiation potential of ASCs at a specific passage number (P5). Subsequent experiments investigated how ASC mechanical properties change over time in culture. We examined ASC elastic and viscoelastic properties at P3–P5 to determine the extent of any changes, as well as their potential impact on cell sorting

Table 1. Mechanical properties of ASCs change as a function of passage number

Passage	$E_{elastic}$ (Pa)	E_{equil} (Pa)	E_0 (Pa)	E_R (Pa)	μ_{app} (Pa·s)	Cell height (μm)
P3	345 ± 150	57 ± 34	206 ± 79	61 ± 33	321 ± 278	12.8 ± 4.8
P4	884 ± 497	79 ± 78	523 ± 292	86 ± 69	734 ± 636	15.6 ± 3.7
P5	800 ± 511	270 ± 226	583 ± 455	292 ± 254	1,690 ± 1,899	8.9 ± 3.7

The mechanical properties of P3, P4 and P5 ASCs were measured using AFM, as described previously.[20] We tested a minimum of 18 cells per passage. Statistical significance between the mechanical properties from P3, P4 and P5 ASCs was determined by one-factor ANOVA with Fisher's LSD post-hoc test ($p < 0.05$). Differences in $E_{elastic}$ and E_0 were significant between P3 and P4, but not P5, ASCs. However, differences in E_{equil}, E_R and cell height were not significant between P3 and P4 ASCs but were significant between those passage groups and P5 ASCs. μ_{app} increased with passage number, and the observed differences across all passages were significant. Data are shown as arithmetic mean ± standard deviation.

approaches. Results indicated that the mechanical properties of spherical ASCs differ significantly by passage number (**Table 1**). Specifically, P3 ASCs were more compliant and less viscous than P4 and P5 ASCs. Increased passage resulted in a less compliant phenotype. These findings suggest that the amount of time ASCs are exposed to environmental signals in their culture environment could be strongly affecting their mechanical properties and their differentiation potential as well.

During their expansion, ASCs are adhered to a rigid plastic substrate and surrounded by a specialized media cocktail composed of nutrients and soluble growth factors. These two environmental stimuli, substrate rigidity and bioactive molecules, are known to play a role in the differentiation potential of MSCs, including ASCs. Previous studies in which MSCs were cultured on substrates that mimicked tissue-specific elasticity showed that substrate compliance could direct stem cell differentiation.[33] Regarding the effects of passage number on ASC differentiation potential, previous studies show that ASCs expanded in monolayer cultures, but in the presence of chondrogenic-inducing soluble factors like bone morphogenic protein 6, enhanced ASC chondrogenic potential.[34] Interestingly, culturing and passaging mature chondrocytes in these two-dimensional conditions leads to their dedifferentiation, which occurs concomitantly with an increase in the cells' viscoelastic moduli.[35] However, the phenotype of these dedifferentiated chondrocytes can be partially rescued if they are re-seeded on a micropatterned, two-dimensional substrate that imparts the cells with a round morphology.[36] In the end, passage number is an important factor in predicating stem cell fate because it is associated with how long ASCs are exposed to chemical and mechanical signals, which not only elicit biological changes, but also mechanical property changes.

Implications and Future Directions

In this report, we provided an overview of the role cellular mechanical properties play in predicting ASC differentiation potential and showed that these mechanical properties can change as a function of time and passage. The collective findings of the aforementioned studies provide evidence that cellular mechanical properties can be used as label-free biomarkers to sort ASCs displaying lineage-specific preferences. By gaining an understanding of the factors that influence cellular mechanical properties, we can better understand how these properties might be used for sorting ASCs from heterogeneous populations. Once such knowledge is obtained, large-scale deformation based sorting devices could be developed to sort large numbers of cells for tissue-specific applications.[37] This approach is potentially revolutionary because sorting would not rely on any kind of labeling but instead on the intrinsic mechanical properties of cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Hetal V. Desai for providing insightful advice. This work was supported in part by National Institutes of Health grants AR054673 and GM104937.

References

1. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7:211-28; PMID:11304456; http://dx.doi.org/10.1089/107632701300062859
2. Guilak F, Lott KE, Awad HA, Cao Q, Hicok KC, Fermor B, et al. Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. J Cell Physiol 2006; 206:229-37; PMID:16021633; http://dx.doi.org/10.1002/jcp.20463
3. Mizuno H, Tobita M, Uysal AC. Concise review: Adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells 2012; 30:804-10; PMID:22415904; http://dx.doi.org/10.1002/stem.1076
4. Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, et al. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005; 54:132-41; PMID:16237275; http://dx.doi.org/10.2302/kjm.54.132
5. Yañez R, Lamana ML, García-Castro J, Colmenero I, Ramírez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells 2006; 24:2582-91; PMID:16873762; http://dx.doi.org/10.1634/stemcells.2006-0228
6. Fang B, Song Y, Liao L, Zhang Y, Zhao RC. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. Transplant Proc 2007; 39:3358-62; PMID:18089385; http://dx.doi.org/10.1016/j.transproceed.2007.08.103
7. Choi JH, Gimble JM, Lee K, Marra KG, Rubin JP, Yoo JJ, et al. Adipose tissue engineering for soft tissue regeneration. Tissue Eng Part B Rev 2010; 16:413-26; PMID:20166810; http://dx.doi.org/10.1089/ten.teb.2009.0544
8. Levi B, Longaker MT. Concise review: adipose-derived stromal cells for skeletal regenerative medicine. Stem Cells 2011; 29:576-82; PMID:21305671; http://dx.doi.org/10.1002/stem.612
9. Beane OS, Darling EM. Isolation, characterization, and differentiation of stem cells for cartilage regeneration. Ann Biomed Eng 2012; 40:2079-97; PMID:22907257; http://dx.doi.org/10.1007/s10439-012-0639-8
10. Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. Stem Cell Res Ther 2010; 1:19; PMID:20587076; http://dx.doi.org/10.1186/scrt19

11. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, Aiba-Kojima E, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J Cell Physiol 2006; 208:64-76; PMID:16557516; http://dx.doi.org/10.1002/jcp.20636
12. Lennon DP, Haynesworth SE, Arm DM, Baber MA, Caplan AI. Dilution of human mesenchymal stem cells with dermal fibroblasts and the effects on in vitro and in vivo osteochondrogenesis. Dev Dyn 2000; 219:50-62; PMID:10974671; http://dx.doi.org/10.1002/1097-0177(2000)9999:9999<::AID-DVDY1037>3.0.CO;2-7
13. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001; 189:54-63; PMID:11573204; http://dx.doi.org/10.1002/jcp.1138
14. Varma MJ, Breuls RG, Schouten TE, Jurgens WJ, Bontkes HJ, Schuurhuis GJ, et al. Phenotypical and functional characterization of freshly isolated adipose tissue-derived stem cells. Stem Cells Dev 2007; 16:91-104; PMID:17348807; http://dx.doi.org/10.1089/scd.2006.0026
15. Zimmerlin L, Donnenberg VS, Pfeifer ME, Meyer EM, Péault B, Rubin JP, et al. Stromal vascular progenitors in adult human adipose tissue. Cytometry A 2010; 77:22-30; PMID:19852056.
16. Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 2008; 102:77-85; PMID:17967785; http://dx.doi.org/10.1161/CIRCRESAHA.107.159475
17. Alt E, Yan Y, Gehmert S, Song YH, Altman A, Gehmert S, et al. Fibroblasts share mesenchymal phenotypes with stem cells, but lack their differentiation and colony-forming potential. Biol Cell 2011; 103:197-208; PMID:21332447; http://dx.doi.org/10.1042/BC20100117
18. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, et al. Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 2006; 24:376-85; PMID:16322640; http://dx.doi.org/10.1634/stemcells.2005-0234
19. Suresh S. Biomechanics and biophysics of cancer cells. Acta Biomater 2007; 3:413-38; PMID:17540628; http://dx.doi.org/10.1016/j.actbio.2007.04.002
20. Darling EM, Topel M, Zauscher S, Vail TP, Guilak F. Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes. J Biomech 2008; 41:454-64; PMID:17825308; http://dx.doi.org/10.1016/j.jbiomech.2007.06.019
21. González-Cruz RD, Fonseca VC, Darling EM. Cellular mechanical properties reflect the differentiation potential of adipose-derived mesenchymal stem cells. Proc Natl Acad Sci U S A 2012; 109:E1523-9; PMID:22615348; http://dx.doi.org/10.1073/pnas.1120349109
22. Darling EM, Zauscher S, Block JA, Guilak F. A thin-layer model for viscoelastic, stress-relaxation testing of cells using atomic force microscopy: do cell properties reflect metastatic potential? Biophys J 2007; 92:1784-91; PMID:17158567; http://dx.doi.org/10.1529/biophysj.106.083097
23. Trickey WR, Vail TP, Guilak F. The role of the cytoskeleton in the viscoelastic properties of human articular chondrocytes. J Orthop Res 2004; 22:131-9; PMID:14656671; http://dx.doi.org/10.1016/S0736-0266(03)0150-5
24. Verstraeten VL, Renes J, Ramaekers FC, Kamps M, Kuijpers HJ, Verheyen F, et al. Reorganization of the nuclear lamina and cytoskeleton in adipogenesis. Histochem Cell Biol 2011; 135:251-61; PMID:21350821; http://dx.doi.org/10.1007/s00418-011-0792-4
25. Titushkin I, Cho M. Modulation of cellular mechanics during osteogenic differentiation of human mesenchymal stem cells. Biophys J 2007; 93:3693-702; PMID:17675345; http://dx.doi.org/10.1529/biophysj.107.107797
26. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 2004; 6:483-95; PMID:15068789; http://dx.doi.org/10.1016/S1534-5807(04)00075-9
27. Rodríguez JP, González M, Ríos S, Cambiazo V. Cytoskeletal organization of human mesenchymal stem cells (MSC) changes during their osteogenic differentiation. J Cell Biochem 2004; 93:721-31; PMID:15660416; http://dx.doi.org/10.1002/jcb.20234
28. Azeloglu EU, Costa KD. Atomic force microscopy in mechanobiology: measuring microelastic heterogeneity of living cells. Methods Mol Biol 2011; 736:303-29; PMID:21660735; http://dx.doi.org/10.1007/978-1-61779-105-5_19
29. Yourek G, Hussain MA, Mao JJ. Cytoskeletal changes of mesenchymal stem cells during differentiation. ASAIO J 2007; 53:219-28; PMID:17413564; http://dx.doi.org/10.1097/MAT.0b013e31802deb2d
30. Wall ME, Bernacki SH, Loboa EG. Effects of serial passaging on the adipogenic and osteogenic differentiation potential of adipose-derived human mesenchymal stem cells. Tissue Eng 2007; 13:1291-8; PMID:17518709; http://dx.doi.org/10.1089/ten.2006.0275
31. Estes BT, Wu AW, Storms RW, Guilak F. Extended passaging, but not aldehyde dehydrogenase activity, increases the chondrogenic potential of human adipose-derived adult stem cells. J Cell Physiol 2006; 209:987-95; PMID:16972251; http://dx.doi.org/10.1002/jcp.20808
32. Estes BT, Diekman BO, Guilak F. Monolayer cell expansion conditions affect the chondrogenic potential of adipose-derived stem cells. Biotechnol Bioeng 2008; 99:986-95; PMID:17929321; http://dx.doi.org/10.1002/bit.21662
33. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126:677-89; PMID:16923388; http://dx.doi.org/10.1016/j.cell.2006.06.044
34. Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. Arthritis Rheum 2006; 54:1222-32; PMID:16572454; http://dx.doi.org/10.1002/art.21779
35. Darling EM, Pritchett PE, Evans BA, Superfine R, Zauscher S, Guilak F. Mechanical properties and gene expression of chondrocytes on micropatterned substrates following dedifferentiation in monolayer. Cell Mol Bioeng 2009; 2:395-404; PMID:20625462; http://dx.doi.org/10.1007/s12195-009-0077-3
36. Darling EM, Athanasiou KA. Rapid phenotypic changes in passaged articular chondrocyte subpopulations. J Orthop Res 2005; 23:425-32; PMID:15734258; http://dx.doi.org/10.1016/j.orthres.2004.08.008
37. Gossett DR, Tse HT, Lee SA, Ying Y, Lindgren AG, Yang OO, et al. Hydrodynamic stretching of single cells for large population mechanical phenotyping. Proc Natl Acad Sci U S A 2012; 109:7630-5; PMID:22547795; http://dx.doi.org/10.1073/pnas.1200107109