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Analysis of the material properties of early chondrogenic differentiated adipose-derived stromal cells (ASC) using an *in vitro* three-dimensional micromass culture system

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Abstract

Cartilage is an avascular tissue with only a limited potential to heal and chondrocytes *in vitro* have poor proliferative capacity. Recently, adipose-derived stromal cells (ASC) have demonstrated a great potential for application to tissue engineering due to their ability to differentiate into cartilage, bone, and fat. In this study, we have utilized a high density three-dimensional (3D) micromass model system of early chondrogenesis with ASC. The material properties of these micromasses showed a significant increase in dynamic and static elastic modulus during the early chondrogenic differentiation process. These data suggest that the 3D micromass culture system represents an *in vitro* model of early chondrogenesis with dynamic cell signaling interactions associated with the mechanical properties of chondrocyte differentiation.

Keywords: Chondrocyte; Mesenchymal stem cells; High density culture; Cartilage repair; Mechanical property; Tissue regeneration; Nanoindentation

Chondrogenesis is a well-orchestrated, multicellular process driven by chondrogenic progenitors that undergo mesenchymal condensation, proliferation, chondrocyte differentiation and maturation [1,2]. The environment in which the chondro-progenitors reside during this process is crucial in establishing chondrogenesis *in vitro* [3,4]. Studies using bone marrow-derived stromal cells (BMSCs) and articular chondrocytes have achieved chondrogenesis in three-dimensional (3D) culture using either a micromass technique, pellet culture, agarose suspension culture, or by seeding cells into a matrix platform such as alginate beads or a PLGA scaffold [5–10]. Most recently we have demonstrated that ambient oxygen is a critical micro-environmental regulator during chondrogenic differentiation and influences the fate of chondrocyte [11].

Adipose-derived stromal cells (ASC) are a promising cell source for tissue engineering because of their abundant supply and their capacity for differentiating to bone, cartilage, muscle and fat [12–16]. ASC are readily accessible, easily expandable and have low donor site morbidity; therefore, applying ASC as an alternative cell source for cartilage repair is an area of intense interest [17–19]. Most of our current research is focusing on studying the chondrogenic and osteogenic capacities of these cells [11,16,20–23]. Because of the low proliferation capability of explanted chondrocytes our group and others are investigating several *in vitro* chondrogenesis differentiation systems using ASC.

Recently, the nanomechanical properties (e.g. hardness and elastic modulus) have been used to measure the material properties of calcified biological tissues [24,25]. Therefore, nanoindentation as a high-resolution probe has received a great deal of attention and provided a

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method for determining material properties on a highly reduced scale [25,26]. With this technique, direct physical property measurements of heterogeneous biological materials, such as friction, adhesion and elasticity, can be determined with high spatial resolution. In addition, the time-dependent mechanical properties of these materials can be obtained in aqueous environments, providing a means for property determination that approximates the *in situ* tissue environment. In particular, cartilage behaves mechanically as a viscoelastic solid due to its composition and structure [27–29]. The mechanical properties of cartilage, such as elastic modulus, depend on the rate of strains which are controlled by the amount of extracellular matrix (ECM) formation, collagen deposition and degradation.

In this study, we utilized a 3D micromass culture system of ASC to create a structural micro-environment for these cells undergoing early chondrogenic differentiation. Subsequently, we have observed the significant differentiation dynamics through the material properties of the differentiated micromasses. We conclude that this *in vitro*, 3D micromass system will be useful for elucidating the mechanism(s) underlying early chondrocyte differentiation and that nanoindentation is a powerful tool to evaluate the material properties of cartilage/chondrocytes during early chondrogenic differentiation.

Materials and methods

Chemicals and media. Dulbecco's modified Eagle's medium (DMEM) media was purchased from Mediatech, Inc. (Herndon, VA). Fetal bovine serum (FBS) was purchased from Omega Scientific, Inc (Tarzana, CA). Other chemicals were from Sigma–Aldrich (St. Louis, MO). ITS premix was from BD Biosciences (Bedford, MA), and TGF-β1 was from Research and Diagnosis, Inc. (Concord, MA).

Animals and AMC harvesting. All experiments were performed in accordance with Stanford University Animal Care and Use Committee guidelines. FVB mice were purchased from Charles River Laboratories Inc. (Wilmington, MA). The inguinal fat pads from three-week old FVB mice were carefully dissected and washed sequentially in a betadine and a phosphate buffered saline (Fisher Scientific, New Jersey, NJ) solution. The hip cartilage tissue (used for the positive control of chondrocytes) from the same mice ($N = 4$) was isolated and snap frozen samples were embedded for histology. The fat pads were finely minced and digested with 0.075% collagenase A (Sigma–Aldrich, St. Louis, MO)/Hanks's Balanced Salt Solution (HBSS) with vigorous shaking in a 37 °C water bath for 30 min. The collagenase A was neutralized with an equal volume of growth media containing DMEM (Mediatech, Inc., Herndon,VA) and 10% FBS. The cell suspension was filtered with a cell strainer (Falcon, 100 μm pore size) to remove larger, undigested fat tissue. After filtration, the cells were pelleted by centrifugation and resuspended in fresh growth media. In approximately two days, cells became confluent and were passaged by trypsin/EDTA.

In vitro chondrogenic experimental design. The micromass technique was modified from Ahrens et al. [4,11,20]. Briefly, passage two cells were trypsinized and resuspended in growth media at a density of 100,000 cells/10 μl. Ten-microliter droplets were seeded in culture dishes and allowed to form cell aggregates and substratum at 37 °C for two and half hours. Chondrogenic media (containing DMEM, 1% FBS, 1% penicillin/streptomycin, 37.5 μg/ml ascorbate-2-phosphate, ITS premix, and 10 ng/ml TGF-β1) was carefully added around the cell aggregates. The chondrogenic media was replenished every three days (Fig. 1).

Micromass harvesting. For the measurement of material properties, micromasses ($N = 4$) were carefully harvested at day 3, day 6, day 9, day 12 and day 15. In order to maintain the properties of the differentiated tissue, the micromasses were maintained in PBS at 4 °C until they were loaded onto the platform of nanoindentation. Micromass slides were stained with hematoxylin and eosin (H&E) and for alcian blue to evaluate the chondrogenic differentiation (data not shown). After observing the consistent chondrogenic differentiation results as reported our previous publications [11,20], we then proceeded with the nanoindentation experiments with these micromasses.

Material properties of differentiated micromasses. In the present experiments, the conventional atomic force microscope (AFM) head was replaced by an electrostatic operated transducer that allowed for topographic imaging as well as local indentation capability (TriboScope nanoindenter, Hysitron, Minneapolis, MN, USA) and mounted on a multimode AFM controlled by NanoScope IIIa electronics (Veeco, Santa Barbara, CA). In the indentation mode, the electrostatic force acting on the spring-suspended center plate of the force–displacement transducer allows the detecting of the time dependence force behavior for prescribed displacement. The detailed description of the instruments has been previously published [25,26]. All experiments were performed in a fluid cell encapsulating samples. A quartz sphere, glued to tungsten rode that is connected to the middle plate of the transducer (Fig. 2A and B) acts as an indenter. The samples, sphere and portion of the tungsten rod were immersed in water during the experiments.

The typical force–displacement curve for cartilage sample is shown in Fig. 3A using a 164 μm sphere radius. The results were analyzed based on Kelvin model:

$$F(t) = S\left[1 - \left(1 - \frac{\tau_\sigma}{\tau_\varepsilon}\right) \cdot \exp\left(-\frac{t}{\tau_\varepsilon}\right)\right] \cdot d(t) \tag{1}$$

where $F(t)$, $d(t)$, S, and t are applied force, displacement, stiffness, and time, respectively. The moduli are calculated from Hertz formula for sphere with radius R:

$$E_S^{\text{Hertz}} = \sqrt{\frac{S^3(1 - V_S^2)^2}{6RF}} \tag{2}$$

where Poisson's ratio, v, is assumed 0.5.

Statistical analysis. At each time point, micromass samples ($N = 4$) were measured and mean and SD were calculated. The statistical analysis was performed using Student's t test. Material property modulus differences were measured using an ANOVA and *post-hoc* multiple comparison test. With all tests, $^*p \leqslant 0.01$ was considered statistically significant.

Results

Micromass formation, histology and staining for chondrogenesis

By 24 h, the aggregates generally coalesced and became spherical (Fig. 1A and B). The micromasses condensed as early chondrogenesis proceeded (Fig. 1C). At later chondrogenesis (day 12 and day 15), micromasses condensed significantly (data not shown). Beginning at day three, the ECM composition between the cells increased markedly and the micromass diameter decreased throughout differentiation, compared to the day 1 micromass (Fig. 1C). We have previously reported the histology and ECM analysis of the condensed micromasses [11,20]. Hematoxylin and Eosin (H&E) staining indicated the structure of the micromasses during this chondrogenic process; alcian blue staining indicated

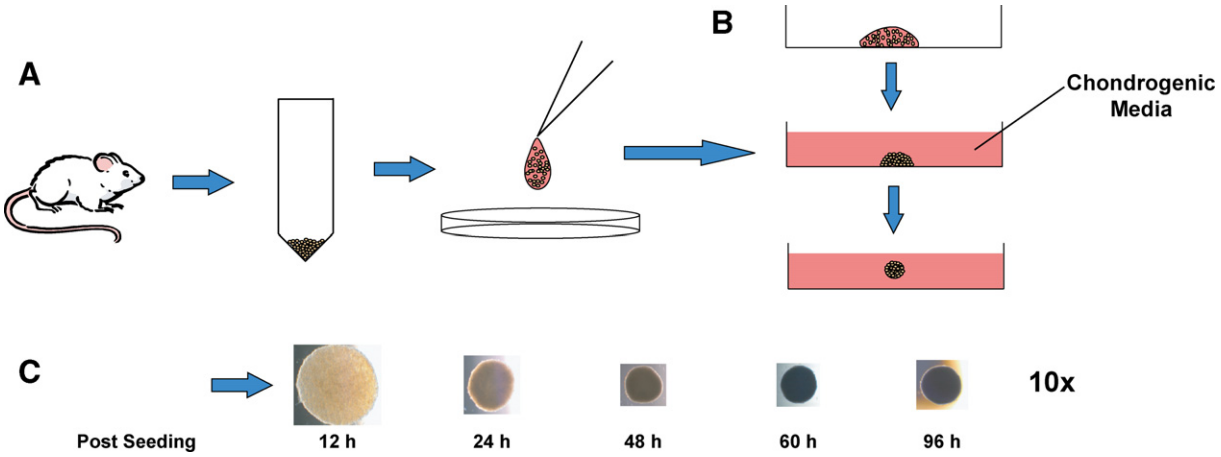

Fig. 1. Schematic process of experimental design for 3D micromasses creation (A) Schematic for cell harvest and high density seeding technique: mouse inguinal fat pads were harvested, digested, and pelleted. Cells were resuspended in culture media at a density of 100,000 cells/10 μl; 10 μl droplets were placed in tissue culture dishes and allowed to dry for 2 h. (B) Schematic for micromass formation: chondrogenic media containing TGF-β1 at 10 ng/ml was added to the cell aggregates. Aggregates condensed and formed spheroids within 24 h of chondrogenic media addition. (C) Microscopic view of cell aggregates and condensation of 3D micromasses. The micromasses are shown post-seeding from 12 to 96 h of condensation and aggregation in chondrogenic culture (magnification 10×).

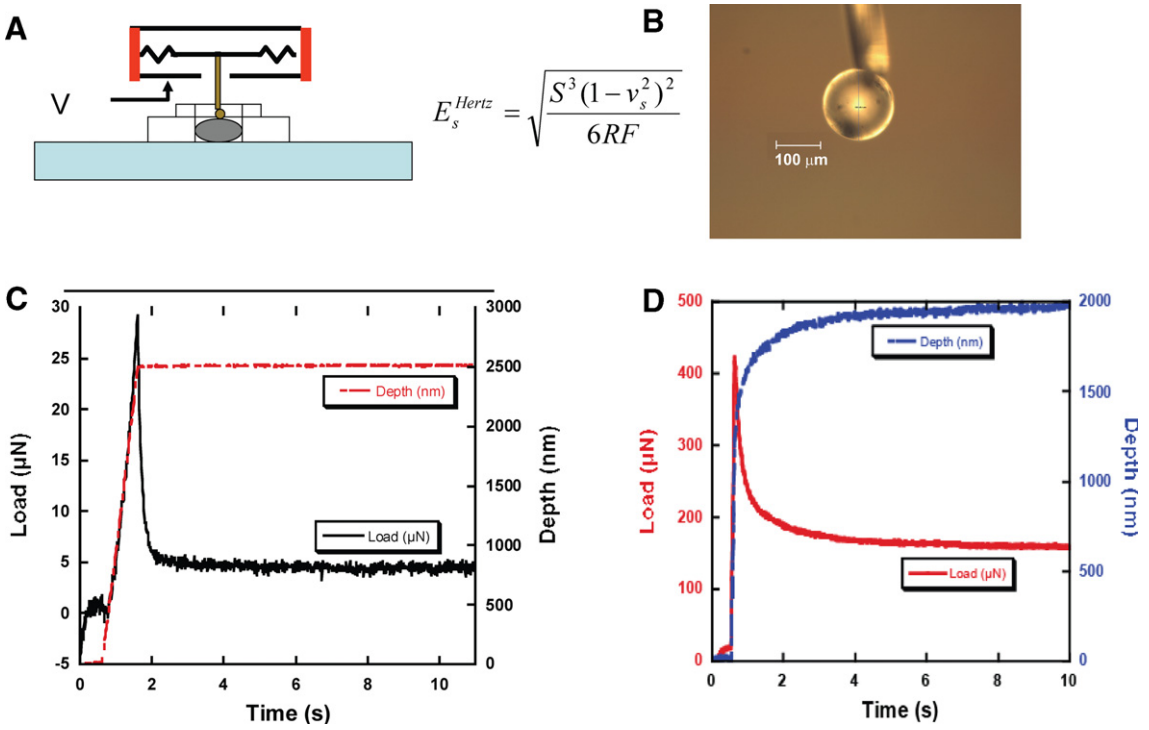

Fig. 2. Nanoindentation of the differentiated micromasses (A) Schematic of indentation technique to measure material properties of micromasses. (B) A digital picture of the glass bead attached to the diamond tip. (C) Theoretical load displacement curves from micromasses. Displacement is shown in red, load is shown in black. (D) Experimental load vs. time curves indicating the error associated with the modulus calculations. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

progressively intense staining over time and suggested proteoglycan accumulation within the micromasses. In addition, staining for collagen II, a main ECM component of chondrogenesis which forms the bulk of the ECM fiber network, was correlated with the alcian blue staining. Mouse fat pads and hips were analyzed as negative and positive controls, respectively (data not shown).

Material properties of micromasses

To determine the differences in mechanical properties of native cartilage (as a positive control) and differentiated micromasses, we employed Atomic Force Microscopy-based (AFM) nanoindentation on micromasses at days 3, 6, 9, 12, and 15. By attaching a glass spherical bead

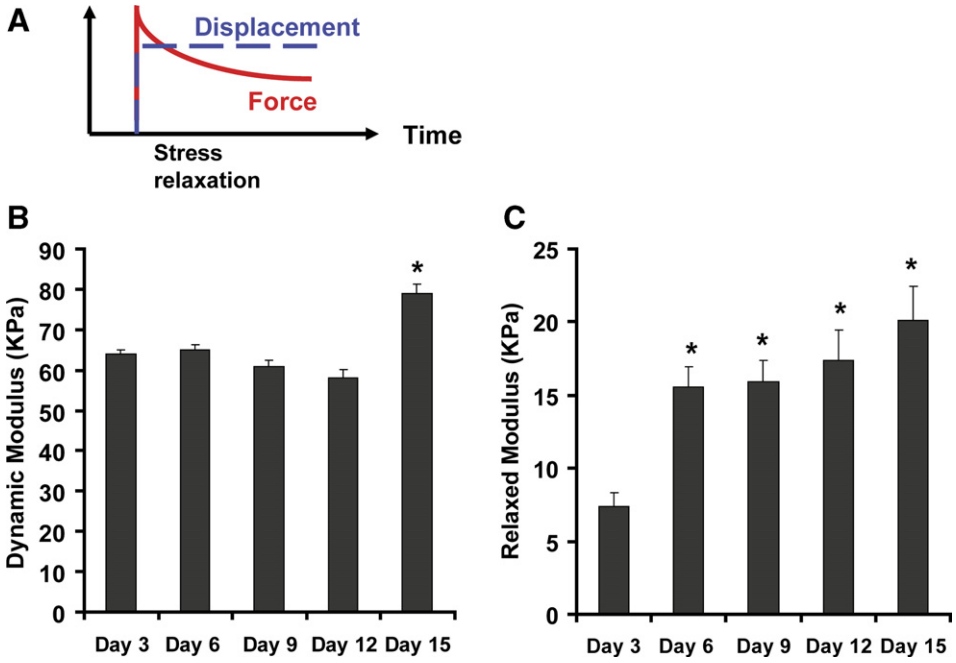

Fig. 3. Changes of material properties during micromass differentiation. (A) The theoretical load displacement curve used to determine the relaxed modulus of the micromasses. The force (red) and displacement (blue) are used to determine the stiffness. (B) The dynamic modulus, and (C) the relaxed modulus of micromasses at days 3, 6, 9, 12, and 15. A significant increase in dynamic modulus ($^{*}p < .001$) is seen at day 15 compare to day 3. The relaxation moduli values showed significant differences at days 6, 9, 12, and 15 compared to day 3 relaxed modulus ($^{*}p < .001$). (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2B) to a diamond pyramid-shaped nanoindentation tip (Fig. 2A), indentations were done on the chondrogenic-differentiated micromasses enabling us to measure the material properties based on the load displacement curves of the micromasses (Fig. 2C). The strain and stress relaxation were determined by applying Kelvin model (Fig. 2C and D).

The dynamic and relaxed modulus of the micromasses significantly increased with time. At later time points (day 6, 9, 12, and 15), significant inductions of the relaxed modulus were observed compared to the early differentiated micromasses suggesting greater changes occurred after day 3 micromass differentiation (Fig. 3C). Relaxed moduli values were 3–5 times lower than dynamic moduli, possibly due to the ability of water to flow through the organic structure. Therefore, the increase in material properties may reflect a greater degree of chondrogenic differentiation within the 3D structure. A significant increase in relaxed modulus was seen at days 6–15 compared to only day 15 in dynamic modulus, possibly due to the highly viscoelastic nature of the micromasses. Indentations on adult mouse femur head (mature cartilage) revealed a modulus of 1.27 KPa, 10-fold larger than that of the micromasses at day 15 (data not shown).

Discussion

Articular cartilage in adults has limited capacity for self-repair following injury. Autologous chondrocyte transplantation is a cell-based orthobiologic technology used for the treatment of cartilage defects [30]. Surgical therapeutic efforts to treat cartilage defects have focused on delivering new, *in vitro*-cultured cells capable of chondrogenesis into the lesions [31]. However, the poor proliferative potential of the chondrocytes *in vitro* has been a major obstacle for stem cell research and the development of clinical therapies. Much of the current research is focused on developing strategies to improve chondrocyte culture expansion and selection [20,31]. Therefore, using ASC, a readily available and abundant potential source of mesenchymal stem cells is a promising approach for cartilage repair/regeneration.

The influence of mechanical stimuli within micro-environments has been extensively described as a large contributor to chondrogenic differentiation *in vitro*. Chondrocytes appear to maintain their phenotype in a 3D culture [5,32]. Consistent with previous reports, we have achieved successful early chondrogenic differentiation in ASC by using the 3D micromass culture system [11,20]. Alcian blue and collagen II staining indicated the progression of chondrogenesis in the 3D micromasses. In addition to the histology, we have assessed sulfated glycosaminoglycan (sGAG) quantities to evaluate this micromass culture system [33]. Gene expression data demonstrated that ASC underwent early chondrogenesis and obtained the characteristics of prechondrocytes: such as significantly higher levels of Sox-9, the critical transcription factor of chondrogenesis; elevated aggrecan and type II collagen, major components of chondrogenesis [11,20]. Collectively, these analyses provide details of spatial–temporal expression of molecules

regulating chondrogenic differentiation in ASC micromasses.

Building on our previous reported changes in histology and marker genes, in this study we also observed significant changes in material properties of the differentiated micromasses. Lacking the technological capabilities of high spatial resolution, analysis of the mechanical properties of differentiated mesenchymal stem cells is still in its infancy. However, in this study, by using a spherical glass bead tip in AFM nanoindentation, we were able to increase the surface area while not compromising the high spatial resolution of the nanoindentation technique. Furthermore, the use of nanoindentation allowed us to assess the properties of these micromasses in nano-scale measurements. Therefore, a significant increase of mechanical integrity was observed over the time of differentiation. Our data indicated that the surrounding micro-environmental factors combined with the 3D structure provide appropriate stimuli and create a niche for early ASC chondrogenesis.

ASC are a heterogeneous cell population consisting of cells in various stages of differentiation and levels of potency. Defining which cells within this population differentiate along specific lineages is a central goal of our research. Factors involved in the regulation of early chondrogenesis will be further determined by utilizing the *in vitro* 3D chondrogenic system. Novel methods of priming and selecting for chondro-progenitors within the heterogeneous population of ASC will contribute to translational therapies utilizing ASC for cartilage repair/replacement. In conclusion, we report that a 3D *in vitro* micromass system represents a dynamic multi-cellular model of early chondrogenesis associated with the mechanical properties of early chondrocyte differentiation.

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References

[1] S. Provot, E. Schipani, Molecular mechanisms of endochondral bone development, Biochem. Biophys. Res. Commun. 328 (2005) 658–665.
[2] B.R. Olsen, A.M. Reginato, W. Wang, Bone development, Annu. Rev. Cell. Dev. Biol. 16 (2000) 191–220.
[3] R. Quarto, G. Campanile, R. Cancedda, B. Dozin, Modulation of commitment, proliferation, and differentiation of chondrogenic cells in defined culture medium, Endocrinology 138 (1997) 4966–4976.
[4] P.B. Ahrens, M. Solursh, R.S. Reiter, Stage-related capacity for limb chondrogenesis in cell culture, Dev. Biol. 60 (1977) 69–82.
[5] H.A. Awad, M.Q. Wickham, H.A. Leddy, J.M. Gimble, F. Guilak, Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds, Biomaterials 25 (2004) 3211–3222.
[6] H.A. Awad, Y.D. Halvorsen, J.M. Gimble, F. Guilak, Effects of transforming growth factor beta1 and dexamethasone on the growth and chondrogenic differentiation of adipose-derived stromal cells, Tissue Eng. 9 (2003) 1301–1312.
[7] D.A. Lee, T. Reisler, D.L. Bader, Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype compared to conventional monolayer techniques, Acta. Orthop. Scand. 74 (2003) 6–15.
[8] J. Chen, C. Wang, S. Lu, J. Wu, X. Guo, C. Duan, L. Dong, Y. Song, J. Zhang, D. Jing, L. Wu, J. Ding, D. Li, In vivo chondrogenesis of adult bone-marrow-derived autologous mesenchymal stem cells, Cell Tissue Res. 319 (2005) 429–438.
[9] K. Uematsu, K. Hattori, Y. Ishimoto, J. Yamauchi, T. Habata, Y. Takakura, H. Ohgushi, T. Fukuchi, M. Sato, Cartilage regeneration using mesenchymal stem cells and a three-dimensional poly-lactic-glycolic acid (PLGA) scaffold, Biomaterials 26 (2005) 4273–4279.
[10] A. Muraglia, A. Corsi, M. Riminucci, M. Mastrogiacomo, R. Cancedda, P. Bianco, R. Quarto, Formation of a chondro-osseous rudiment in micromass cultures of human bone-marrow stromal cells, J. Cell Sci. 116 (2003) 2949–2955.
[11] P. Malladi, Y. Xu, M. Chiou, A.J. Giaccia, M.T. Longaker, Effect of reduced oxygen tension on chondrogenesis and osteogenesis in adipose-derived mesenchymal cells, Am. J. Physiol. Cell Physiol. 290 (2006) C1139–C1146.
[12] J.M. Gimble, M.E. Nuttall, Bone and fat: old questions, new insights, Endocrine 23 (2004) 183–188.
[13] F. Guilak, H.A. Awad, B. Fermor, H.A. Leddy, J.M. Gimble, Adipose-derived adult stem cells for cartilage tissue engineering, Biorheology 41 (2004) 389–399.
[14] P.A. Zuk, M. Zhu, P. Ashjian, D.A. De Ugarte, J.I. Huang, H. Mizuno, Z.C. Alfonso, J.K. Fraser, P. Benhaim, M.H. Hedrick, Human adipose tissue is a source of multipotent stem cells, Mol. Biol. Cell 13 (2002) 4279–4295.
[15] J.M. Gimble, F. Guilak, Differentiation potential of adipose derived adult stem (ADAS) cells, Curr. Top Dev. Biol. 58 (2003) 137–160.
[16] Y. Xu, P. Malladi, D.R. Wagner, M.T. Longaker, Adipose-derived mesenchymal cells as a potential cell source for skeletal regeneration, Curr. Opin. Mol. Ther. 7 (2005) 300–305.
[17] R. Ogawa, H. Mizuno, H. Hyakusoku, A. Watanabe, M. Migita, T. Shimada, Chondrogenic and osteogenic differentiation of adipose-derived stem cells isolated from GFP transgenic mice, J. Nippon. Med. Sch. 71 (2004) 240–241.
[18] H. Tanaka, C.L. Murphy, C. Murphy, M. Kimura, S. Kawai, J.M. Polak, Chondrogenic differentiation of murine embryonic stem cells: effects of culture conditions and dexamethasone, J. Cell Biochem. 93 (2004) 454–462.
[19] J.I. Huang, P.A. Zuk, N.F. Jones, M. Zhu, H.P. Lorenz, M.H. Hedrick, P. Benhaim, Chondrogenic potential of multipotential cells from human adipose tissue, Plast. Reconstr. Surg. 113 (2004) 585–594.
[20] M. Chiou, Y. Xu, M.T. Longaker, Mitogenic and chondrogenic effects of fibroblast growth factor-2 in adipose-derived mesenchymal cells, Biochem. Biophys. Res. Commun. 343 (2006) 644–652.
[21] P. Malladi, Y. Xu, G.P. Yang, M.T. Longaker, Functions of vitamin D, retinoic Acid, and dexamethasone on mouse adipose-derived mesenchymal cells (AMCs), Tissue Eng. (2005).
[22] C.M. Cowan, Y.Y. Shi, O.O. Aalami, Y.F. Chou, C. Mari, R. Thomas, N. Quarto, C.H. Contag, B. Wu, M.T. Longaker, Adipose-derived adult stromal cells heal critical-size mouse calvarial defects, Nat. Biotechnol. 22 (2004) 560–567.
[23] C.M. Cowan, O.O. Aalami, Y.Y. Shi, Y.F. Chou, C. Mari, R. Thomas, N. Quarto, R.P. Nacamuli, C.H. Contag, B. Wu, M.T. Longaker, Bone morphogenetic protein 2 and retinoic acid accelerate in vivo bone formation, osteoclast recruitment, and bone turnover, Tissue Eng. 11 (2005) 645–658.
[24] N.E. Lane, W. Yao, M. Balooch, R.K. Nalla, G. Balooch, S. Habelitz, J.H. Kinney, L.F. Bonewald, Glucocorticoid-treated mice have localized changes in trabecular bone material properties and osteocyte lacunar size that are not observed in placebo-treated or estrogen-deficient mice, J. Bone Miner Res. 21 (2006) 466–476.

[25] G. Balooch, M. Balooch, R.K. Nalla, S. Schilling, E.H. Filvaroff, G.W. Marshall, S.J. Marshall, R.O. Ritchie, R. Derynck, T. Alliston, TGF-beta regulates the mechanical properties and composition of bone matrix, Proc. Natl. Acad. Sci. USA 102 (2005) 18813–18818.
[26] G. Balooch, G.W. Marshall, S.J. Marshall, O.L. Warren, S.A. Asif, M. Balooch, Evaluation of a new modulus mapping technique to investigate microstructural features of human teeth, J. Biomech. 37 (2004) 1223–1232.
[27] N.M. Bachrach, W.B. Valhmu, E. Stazzone, A. Ratcliffe, W.M. Lai, V.C. Mow, Changes in proteoglycan synthesis of chondrocytes in articular cartilage are associated with the time-dependent changes in their mechanical environment, J. Biomech. 28 (1995) 1561–1569.
[28] B. Cohen, W.M. Lai, V.C. Mow, A transversely isotropic biphasic model for unconfined compression of growth plate and chondro-epiphysis, J. Biomech. Eng. 120 (1998) 491–496.
[29] X. Lux Lu, C. Miller, F.H. Chen, X. Edward Guo, V.C. Mow, The generalized triphasic correspondence principle for simultaneous determination of the mechanical properties and proteoglycan content of articular cartilage by indentation, J. Biomech. (2007).
[30] K. Bobacz, L. Erlacher, J. Smolen, A. Soleiman, W.B. Graninger, Chondrocyte number and proteoglycan synthesis in the aging and osteoarthritic human articular cartilage, Ann. Rheum. Dis. 63 (2004) 1618–1622.
[31] C. Chung, J. Mesa, G.J. Miller, M.A. Randolph, T.J. Gill, J.A. Burdick, Effects of auricular chondrocyte expansion on neocartilage formation in photocrosslinked hyaluronic acid networks, Tissue Eng. 12 (2006) 2665–2673.
[32] A.L. Carlberg, B. Pucci, R. Rallapalli, R.S. Tuan, D.J. Hall, Efficient chondrogenic differentiation of mesenchymal cells in micromass culture by retroviral gene transfer of BMP-2, Differentiation 67 (2001) 128–138.
[33] P. Malladi, Y. Xu, M. Chiou, A. Ciaccia, L. MT., Hypoxia inducibale factor-1 alpha deficiency affects chondrogenesis of adipose-derived adult stromal cells (ADAS), Tissue Eng. (2007). PMID: 17518738.