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Effects of FGF-2 on human adipose tissue derived adult stem cells morphology and chondrogenesis enhancement in Transwell culture

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ABSTRACT

Injured cartilage is difficult to repair due to its poor vascularisation. Cell based therapies may serve as tools to more effectively regenerate defective cartilage. Both adult mesenchymal stem cells (MSCs) and human adipose derived stem cells (hADSCs) are regarded as potential stem cell sources able to generate functional cartilage for cell transplantation. Growth factors, in particular the TGF-b superfamily, influence many processes during cartilage formation, including cell proliferation, extracellular matrix synthesis, maintenance of the differentiated phenotype, and induction of MSCs towards chondrogenesis. In the current study, we investigated the effects of FGF-2 on hADSC morphology and chondrogenesis in Transwell culture. hADSCs were obtained from patients undergoing elective surgery, and then cultured in expansion medium alone or in the presence of FGF-2 (10 ng/ml). mRNA expression levels of SOX-9, aggrecan and collagen type II and type X were quantified by real-time polymerase chain reaction. The morphology, doubling time, trypsinization time and chondrogenesis of hADSCs were also studied. Expression levels of SOX-9, collagen type II, and aggrecan were all significantly increased in hADSCs expanded in presence of FGF-2. Furthermore FGF-2 induced a slender morphology, whereas doubling time and trypsinization time decreased. Our results suggest that FGF-2 induces hADSCs chondrogenesis in Transwell culture, which may be beneficial in cartilage tissue engineering.

1. Introduction

Cartilage damage such as associated with osteoarthritis often exhibits limited repair due to its avascularity. Therefore, finding effective treatments to improve cartilage repair remains a challenge. Strategies based on tissue engineering have acquired substantial attention as possible approaches for cartilage repair [1]. Recent studies on cell-based cartilage tissue engineering have investigated the use of biomaterials seeded with chondrocytes or progenitor cells and/or chondrogenic factors [2]. Adult mesenchymal stem cells (MSCs) such as bone marrow-derived mesenchymal stem cells (BMSCs) have been previously been investigated a new cell sources for cartilage engineering [2–4]. Adipose derived adult stem cells (ADSCs) may provide an additional source of stem cells for chondrogenesis, osteogenesis, and adipogenesis. ADSCs can be obtained easily, with minimally invasive procedures, and in much higher quantities than MSCs derived from bone marrow [5–9]. Human MSCs as well as ADSCs are a heterogeneous population of stem cells with various chondrogenic potential [10,11]. In fact most non-ADSCs (derived from these preparations), including adipose precursor cells, fibroblasts, and endothelial cells, lack chondrogenic potential. Moreover, not all ADSCs exhibit the same chondrogenic potential [12]. Therefore it will be crucial to improve the isolation and expansion efficacy of ADSCs to investigate their possible clinical relevance [1].

When the hADSCs cells were propagated in culture their morphology become flattened and spread out on the culture surface. After three passages proliferation slowed. To overcome this, we applied expansion medium containing TGF-b 1, EGF and b-FGF in very low, but relevant, concentrations [13]. Fibroblast growth factors (FGFs) belong to a family of heparin-binding polypeptide growth factors that have over 20 members, including FGF-2 [14,15]. It has been shown that FGF-2 acts as a regulator in several signaling cascades involved in the development and maintenance of cartilage, especially in early stages of limb development [16]. Recently it has been shown that FGF-2 can enhance cellular

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proliferation and chondrogenic differentiation of hBMSCs [4]. FGF-2 significantly promoted proliferation and chondrogenic potential of mice ADSC in micromass culture. It also increased the mRNA levels of Sox-9 and N-cadherin [17]. In addition to biological agents, the induction of chondrogenesis in ADSCs appears to be enhanced by culture systems that induce a rounded cell shape, including (1) scaffold free systems such as pellet culture and Transwell culture or (2) application of scaffolds [6]. Moreover it has been shown that human bone marrow stem cells (hBMSCs) can undergo chondrogenic differentiation in Transwell culture [18]. We therefore investigated the effects of FGF-2 on the morphology, proliferation and chondrogenesis of hADSCs in Transwell culture.

2. Materials and methods

2.1. Isolation and culture of hADSCs

Human adipose tissue adult stem cells were derived from subcutaneous fat tissue of five individuals, who underwent elective surgery. All procedures were conducted according to Isfahan University of Medical Sciences, Medical Faculty ethic committee approval. Adipose tissue was washed thoroughly with sterile PBS (sigma), finely diced and then digested with 0.1% collagenase A (Sigma) solution for 30–45 min at 37 °C. Collagenase A was neutralized with an equal volume of expansion medium containing DMEM-low glucose (Sigma) with 10% FBS (Invitrogen) and 1% peniciline/streptomycine (Gibco). Following this the suspension was centrifuged, and the cell pellets were planted on culture flasks containing fresh expansion medium. The media was changed on the following day, and thereafter every 3–4 days. The cells were passaged a further two times at which point they were trypsinized and counted using a hemocytometer.

2.2. Cells morphology, doubling time and trypsinization

hADSCs were plated at a density of 8000 cells/cm^2 at each passage. hADSCs were divided into two groups. Both groups were culured in basic expansion medium (DMEM low glucose containing 10% FBS), while in the treatment group FGF-2 (10 ng/ml, Royan Institute) was included in the expansion medium. Cell morphology was photographed using phase-contrast microscopy every day. Doubling time of the third passage was calculated using the algorithm provided by http://www.doubling-time.com [19,20].

For trypsinization, three flasks in each group at various passages were trypsinized with trypsin–EDTA (0.05%, Gibco). The time required to release 100% of cells from the culture flask was measured.

2.3. Chondrogenic differentiation

After passage 3, harvested hADSCs were resuspended in chondrogenic culture medium (high glucose Dulbecco's modified Eagle medium (Gibco), 100 μg sodium pyruvate (Sigma), 10 ng/ml TGF-ß1 (R & D Systems) 100 nM dexamethasone (Sigma), 1% ITS + Premix (sigma), 40 μg/ml proline (Sigma), 50 μg/ml ascorbate-2-phosphate (Sigma) and 1% peniciline–streptomicine (Gibco)). For chondrogenesis in Transwells, aliquots of hADScs (5×10^5 in 100 μl of medium) were pipetted directly onto the dry filter inserts of Transwells in multiwall plates (6.5 mm diameter 0.4-μm pore size polycarbonate membrane, 24 well plate Corning Life Sciences) .The plate was centrifuged at 200 g for 5 min, and 0.5 ml of chondrogenic medium was added to the lower chamber of the filters. The medium of the upper and lower chambers were replaced every 2 days, 100 μl total, for 14 days. For control group the incomplete chondrogenic medium that is the same with the mentioned chondrogenic medium was used except without TGF-ß1.

2.4. RNA isolation and real-time polymerase chain reaction

RNA samples ($n = 3$ per data point) were prepared after14 days of chondrogenic differentiation. Discs were disrupted in liquid nitrogen using a small pestle and then RNA isolated using TRIzol reagents (Invitrogen). The RNA was reverse transcribed using RevertAid First Strand cDNA Synthesis Kit (Fermentase) with oligo dT primers. The real-time polymerase chain reaction was performed using SYBRGreen PCR Master mix (Fermentase) and the StepOne Plus™ quantitative Real time PCR detection System (Applied Biosystems). Primers were designed for each gene using the AlleleID software (Primer Biosoft), which generated the follow sequences: collagen II (forward-CTGGTGATGATGGTGAAG, reverse-CCTGGATAACCTCTGTGA), collagen X (forward-AGAATCCATCTG AGAATATGC, reverse-CCTCTTACTGCTATACCTTTAC), Sox-9 (forward-TTCAGCAGCCAATAAGTG, reverse-GTGGAATGTCTTGAAGGTTA), aggrecan (forward-GTGGGACTGAAGTTCTTG, reverse-GTTGTC ATGGTCTGAAGTT) and GAPDH (forward-AAGCTCATTTCCTGGTAT G-reverse-CTTCTTCTTGTGCTCTTG). The gene of interest was normalized against the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression level of each target gene was calculated as $2^{-\Delta\Delta Ct}$, as previously described [21].

2.5. Statistical analysis

Doubling time and trypsinization time were analyzed using a Student *t*-test and the comparison of gene expression was carried out using a One-way ANOVA analysis of variance and Post hoc (Tukey) analysis. Significant differences were considered those with *P* value of <0.05. All data was reported as mean ± SD.

3. Results

3.1. Histologic changes in response to FGF-2

Following transfer of hADSCs cells to expansion media with or without FGF (10 ng/ml), cells were passaged a further two times. At this point cells in both the control and treatment groups displayed rapid changes in cellular morphology. At early stages, hADSCs passaged without FGF-2 were thin and spindle shaped but gradually became wider and spindle shaped, and at much later stages became large and flat. By contrast, hADSCs passaged in the presence of FGF-2 maintained their thin spindle-shape even at passage 3.

3.2. Trypsinization and doubling time of hADSCs

The presence of FGF-2 in the expansion medium maintained the hADSCs' thin spindle-shape (Fig. 1A), which empirically correlated with an easier detachment from the culture flasks. The trypsinization time for control hADSCs expanded without FGF-2 was 5–6 min (Fig. 2A), significantly higher than the 3–4 min required for the treatment group ($P < 0.01$). FGF-2 also affected the doubling time of ADSCs. At passage 3, the doubling time of the FGF-2 treatment group (mean 1.7 days) was significantly lower than the control group (mean 4.6 days) $P < 0.01$ (Fig. 2B).

3.3. Gene expression changes of hADSCs cultured in Transwell culture

Real-time PCR was used to measure changes in the gene expression profile induced by the inclusion of FGF-2 in chondrogenic media during differentiation. Briefly, Transwell cultures of hADSCs

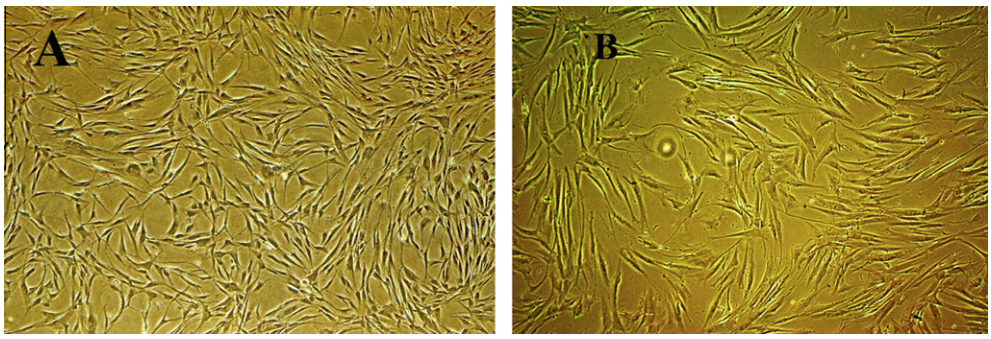

Fig. 1. The morphology of hADSCs that expanded in two different expansion medium. ADSCs that expanded in medium supplemented with FGF-2 (10 ng/ml), the cells are spindle shaped and smaller (A). ADSCs were propagated in ordinary expansion medium, the cells became wider but were still spindle shaped (B).

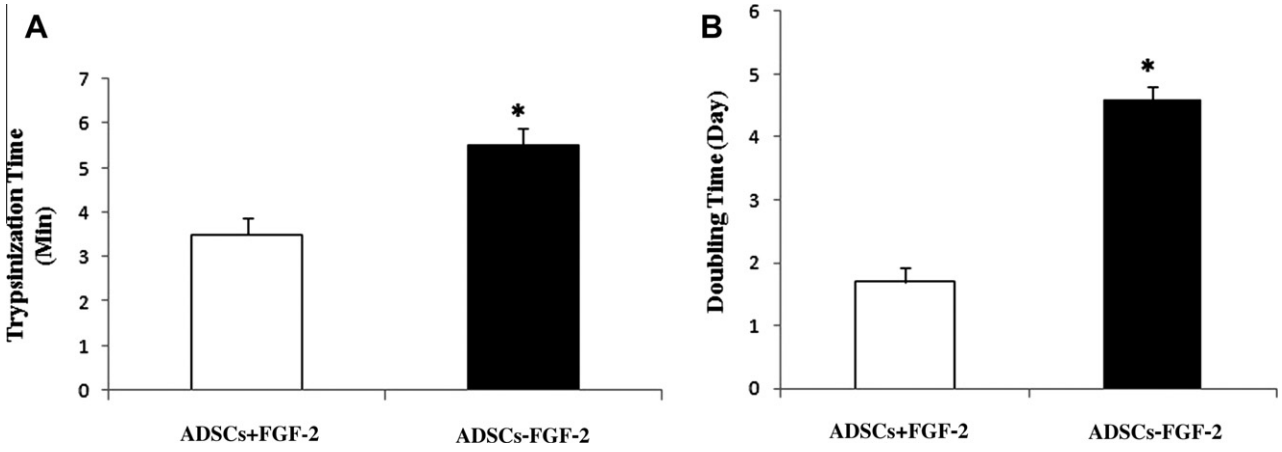

Fig. 2. FGF-2 effects on the doubling time and trypsinization time of ADSCs. Trypsinization time also was affected with FGF-2 in expansion medium. (A) Different expansion medium cause difference in doubling time of ADSCs. (B) All data represented as a mean ± SD. $^{*}p < 0.05$.

supplemented with FGF-2 showed greater chondrogenic gene expression than hADSCs differentiated without FGF-2.

Collagen II expression was significantly up regulated in the treatment group compared to controls. More specifically, the mRNA levels of collagen II found in discs differentiated from FGF-2 treated hADSCs were significantly greater (524-fold) than in discs differentiated from control hADSCs (251-fold, $P < 0.05$) which was significantly higher than discs differentiated from control hADSCs without TGF-1beta.The same trend was seen for the expression of aggrecan and Sox-9. Sox-9 expression was enhanced in discs differentiated from treated hADSCs compared to control hADSCs, both of which were much higher than control hADSCs differentiated in the absence of TGF-1beta (Fig. 3). For aggrecan, discs derived from treated hADSCs showed about 47.7-fold upregulation, compared to a 33.8-fold upregulation in control hADSCs, when normalized to control hADSCs differentiated without TGF-1beta. During chondrogenic differentiation, collagen X is considered an undesirable marker of hypertrophy, and is unfortunately expressed during chondrogenesis in many of culture systems. Encouragingly, in our system, collagen X expression in discs differentiated from FGF-2 treated hADSCs was markedly reduced compared to untreated controls (Fig. 3).

4. Discussion

Repair of cartilage injuries are challenging to orthopedic surgeons, due to cartilage's poor capacity for natural repair. Using autologous adult stem cells such as BMSCs and ADSCs to regenerate defective cartilage may become a viable clinical option. However, characteristics of these cells such as morphology, proliferation rate, trypsinization time, and eventual differentiation capacity may change under prolonged *in vitro* culture [13,22,23]. In this study, we investigated the effects of FGF-2 on these characteristics during passaging and subsequent differentiation in Transwell culture. We demonstrate that FGF-2 reduces the doubling and trypsinization time of hADSCs during passaging, and promotes the expression of chondrogenic marker genes (SOX-9, collagen type II and aggrecan) during subsequent chondrogenic differentiation.

Mesenchymal stem cells (MSCs) have been considered for possible clinical use because of their self-renewing potential and multipotentially. They have been isolated from a variety of tissues including bone marrow [3], muscle [24], and adipose tissue [25]. Among these tissues, bone marrow has been well established as a MSCs source in humans. However, the availability of such tissue, and the recoverable cell numbers are relatively limited [26]. Incidentally, adipose tissue is an interesting MSCs source because of its adequate supply, and because large numbers of MSCs can be obtained from a relatively small amount of fat tissue [27]. Moreover, previous studies and now this current study, demonstrate that MSCs isolated from fat tissue have chondrogenic potential [28]. ADSCs therefore, represent a viable cell source for autologous cartilage tissue engineering.

Previous studies describe adult MSCs as having a large cytoplasmic volume when cultured without bFGF, while fetal and adult bone marrow-derived MSCs cultured with bFGF (5 ng/ml) have a smaller fibroblast-like morphology with a spindle-shaped cytoplasm [35]. Similar observations have been made for human bone

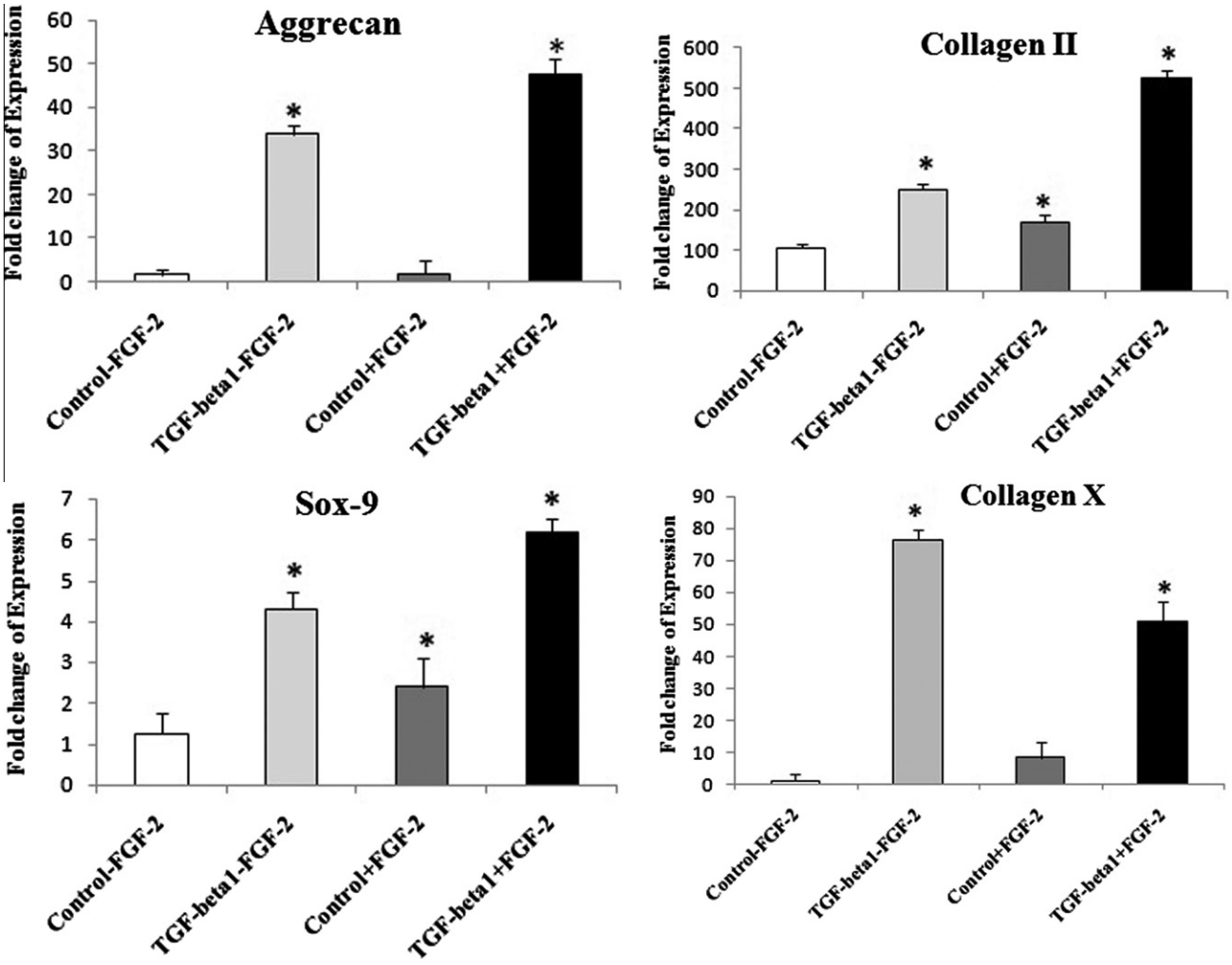

Fig. 3. The relative expression level of collagen II, Sox-9, aggrecan and collagen X after chondrogenesis in Transwell culture and the effects of expansion medium supplemented with FGF-2. All data were normalized to GAPDH expression and compared to the non-FGF-2 expanded control. Values were represented as mean ± SD. $^{*}p < 0.05$.

marrow derived stem cells (hBMSCs) [14,18,36]. Consistent with these reports, we also observe that FGF-2 maintains the small cytoplasmic volume and spindle morphology of hADSCs over repeated passages. This strong effect of FGF-2 on hADSCs morphology suggests it may modulate their growth and differentiation potential. To investigate the possible affect of FGF-2 on the lineage-specific chondrogenic differentiation of hADSCs, primary hADSCs were passaged in the presence or absence of FGF-2 and their chondrogenesis was subsequently assessed using a three-dimensional Transwell culture system [5,7,37]. Induced chondrogenesis in Transwell culture routinely leads to an upregulation of collagen II, Sox-9 and aggrecan [5,7,37].

We demonstrate that passaging of hADSCs in the presence of FGF-2 promotes the expression of these chondrogenic markers upon hADSCs differentiation. SOX-9 is one of the earliest markers expressed in cells undergoing precartilaginous condensation [29] and it is also required for the expression of collagen type II and aggrecan, which are expressed during initial cell condensation and further differentiation into chondrocytes.

It has previously been shown that extended *in vitro* culture of MSCs leads to hypertrophic chondrocytes and expression of collagen type X upon their differentiation [30–32]. In this study, expression levels of collagen type X were lower in discs differentiated from hADSCs cultured in presence of FGF-2, indicating that FGF-2 inhibited the formation of fibrocartilage and degeneration (hypertrophy). Therefore, it can be concluded that *in vivo* passaging hADSCs in the presence of FGF2 improves their chondrogenic differentiation capacity.

We also show that hADSCs cultured in presence of FGF-2 (10 ng/ml) exhibit a lower doubling and trypsinization time (Fig. 1). Growth factors including FGF-2 can modulate mesenchymal cells' growth and differentiation [14,17,33], while FGF-2 in particular can influence self-renewal of ADSCs during propagation *in vitro* [34]. Since the poor proliferation of chondrocytes *in vitro* presents a significant obstacle for cartilage repair, FGF-2 may be a useful tool for expanding chondrogenic progenitor cells to numbers viable for cell replacement.

We conclude that FGF-2 inclusion during routing passaging of hADSCs improves the scalability and efficacy of their subsequent chondrogenesis in Transwell culture. Therefore, FGF-2 may serve as a useful tool for promoting translational research for cell-based therapies targeting cartilage tissue regeneration.

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References

[1] A.M. Handorf, W.J. Li, Fibroblast growth factor-2 primes human mesenchymal stem cells for enhanced chondrogenesis, PLoS One 6 (2011) e22887.
[2] C. Vinatier, D. Mrugala, C. Jorgensen, J. Guicheux, D. Noel, Cartilage engineering: a crucial combination of cells, biomaterials and biofactors, Trends Biotechnol. 27 (2009) 307–314.
[3] A.I. Caplan, S.P. Bruder, Mesenchymal stem cells: building blocks for molecular medicine in the 21st century, Trends Mol. Med. 7 (2001) 259–264.
[4] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, D. Prockop, E. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317.
[5] 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.
[6] 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.
[7] B.T. Estes, A.W. Wu, F. Guilak, Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6, Arthritis Rheum. 54 (2006) 1222–1232.
[8] Z.F. Lu, B. Zandieh Doulabi, P.I. Wuisman, R.A. Bank, M.N. Helder, Differentiation of adipose stem cells by nucleus pulposus cells: configuration effect, Biochem. Biophys. Res. Commun. 359 (2007) 991–996.
[9] D.W. Wang, B. Fermor, J.M. Gimble, H.A. Awad, F. Guilak, Influence of oxygen on the proliferation and metabolism of adipose derived adult stem cells, J. Cell Physiol. 204 (2005) 184–191.
[10] A. Muraglia, R. Cancedda, R. Quarto, Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model, J. Cell Sci. 113 (Pt 7) (2000) 1161–1166.
[11] B. Delorme, J. Ringe, C. Pontikoglou, J. Gaillard, A. Langonne, L. Sensebe, D. Noel, C. Jorgensen, T. Haupl, P. Charbord, Specific lineage-priming of bone marrow mesenchymal stem cells provides the molecular framework for their plasticity, Stem cells 27 (2009) 1142–1151.
[12] T. Jiang, W. Liu, X. Lv, H. Sun, L. Zhang, Y. Liu, W.J. Zhang, Y. Cao, G. Zhou, Potent in vitro chondrogenesis of CD105 enriched human adipose-derived stem cells, Biomaterials 31 (2010) 3564–3571.
[13] B.T. Estes, B.O. Diekman, J.M. Gimble, F. Guilak, Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype, Nat. Protoc. 5 (2010) 1294–1311.
[14] L.A. Solchaga, K. Penick, J.D. Porter, V.M. Goldberg, A.I. Caplan, J.F. Welter, FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells, J. Cell Physiol. 203 (2005) 398–409.
[15] M.A. Dupree, S.R. Pollack, E.M. Levine, C.T. Laurencin, Fibroblast growth factor 2 induced proliferation in osteoblasts and bone marrow stromal cells: a whole cell model, Biophys. J. 91 (2006) 3097–3112.
[16] D.M. Ornitz, P.J. Marie, FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease, Genes Dev. 16 (2002) 1446–1465.
[17] 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.
[18] A.D. Murdoch, L.M. Grady, M.P. Ablett, T. Katopodi, R.S. Meadows, T.E. Hardingham, Chondrogenic differentiation of human bone marrow stem cells in Transwell cultures: generation of scaffold-free cartilage, Stem cells 25 (2007) 2786–2796.
[19] R. Koninckx, A. Daniels, S. Windmolders, F. Carlotti, U. Mees, P. Steels, J.L. Rummens, M. Hendrikx, K. Hensen, Mesenchymal stem cells or cardiac progenitors for cardiac repair? a comparative study, Cell Mol. Life Sci. 68 (2011) 2141–2156.
[20] W. Fiebiger, U. Olszewski, E. Ulsperger, K. Geissler, G. Hamilton, In vitro cytotoxicity of novel platinum-based drugs and dichloroacetate against lung carcinoid cell lines, Clin. Transl. Oncol. 13 (2011) 43–49.
[21] A. Esmaeili, S.R. Zaker, Differential expression of glycine receptor subunit messenger RNA in the rat following spinal cord injury, Spinal cord 49 (2011) 280–284.
[22] M.M. Bonab, K. Alimoghaddam, F. Talebian, S.H. Ghaffari, A. Ghavamzadeh, B. Nikbin, Aging of mesenchymal stem cell in vitro, BMC Cell Biol. 7 (2006) 14.
[23] I.E. Erickson, A.H. Huang, C. Chung, R.T. Li, J.A. Burdick, R.L. Mauck, Differential maturation and structure–function relationships in mesenchymal stem cell- and chondrocyte-seeded hydrogels, Tissue Eng. Part A 15 (2009) 1041–1052.
[24] K.A. Jackson, T. Mi, M.A. Goodell, Hematopoietic potential of stem cells isolated from murine skeletal muscle, Proc. Natl. Acad. Sci. 96 (1999) 14482.
[25] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick, Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng. 7 (2001) 211–228.
[26] B.M. Strem, K.C. Hicok, M. Zhu, I. Wulur, Z. Alfonso, R.E. Schreiber, J.K. Fraser, M.H. Hedrick, Multipotential differentiation of adipose tissue-derived stem cells, Keio J. Med. 54 (2005) 132–141.
[27] D.A. De Ugarte, P.H. Ashjian, A. Elbarbary, M.H. Hedrick, Future of fat as raw material for tissue regeneration, Ann. Plastic Surg. 50 (2003) 215.
[28] Z.J. Liu, Y. Zhuge, O.C. Velazquez, Trafficking and differentiation of mesenchymal stem cells, J. Cell. Biochem. 106 (2009) 984–991.
[29] M.B. Goldring, K. Tsuchimochi, K. Ijiri, The control of chondrogenesis, J. Cell. Biochem. 97 (2006) 33–44.
[30] A. Dickhut, K. Pelttari, P. Janicki, W. Wagner, V. Eckstein, M. Egermann, W. Richter, Calcification or dedifferentiation: requirement to lock mesenchymal stem cells in a desired differentiation stage, J. Cell Physiol. 219 (2009) 219–226.
[31] A. Winter, S. Breit, D. Parsch, K. Benz, E. Steck, H. Hauner, R.M. Weber, V. Ewerbeck, W. Richter, Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells, Arthritis Rheum. 48 (2003) 418–429.
[32] K. Pelttari, A. Winter, E. Steck, K. Goetzke, T. Hennig, B.G. Ochs, T. Aigner, W. Richter, Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice, Arthritis Rheum. 54 (2006) 3254–3266.
[33] M. Presta, P. Dell'Era, S. Mitola, E. Moroni, R. Ronca, M. Rusnati, Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis, Cytokine Growth Factor Rev. 16 (2005) 159–178.
[34] L.E. Zaragosi, G. Ailhaud, C. Dani, Autocrine fibroblast growth factor 2 signaling is critical for self-renewal of human multipotent adipose-derived stem cells, Stem Cells 24 (2006) 2412–2419.
[35] P.V. Guillot, C. Gotherstrom, J. Chan, H. Kurata, N.M. Fisk, Human first-trimester fetal MSC express pluripotency markers and grow faster and have longer telomeres than adult MSC, Stem Cells 25 (2007) 646–654.
[36] S. Hankemeier, M. Keus, J. Zeichen, M. Jagodzinski, T. Barkhausen, U. Bosch, C. Krettek, M.V. Griensven, Modulation of proliferation and differentiation of human bone marrow stromal cells by fibroblast growth factor 2: potential implications for tissue engineering of tendons and ligaments, Tissue Eng. 11 (2005) 41–49.
[37] B. Johnstone, T.M. Hering, A.I. Caplan, V.M. Goldberg, J.U. Yoo, In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells, Exp. Cell Res. 238 (1998) 265–272.