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LABORATORY INVESTIGATIONS

# Comparison of Multipotent Differentiation Potentials of Murine Primary Bone Marrow Stromal Cells and Mesenchymal Stem Cell Line C3H10T1/2

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**Abstract** Murine C3H10T1/2 cells have many features of mesenchymal stem cells (MSCs). Whether or not the multipotent differentiation capability of C3H10T1/2 cells is comparable to that of primary bone marrow–derived MSCs (BM-MSCs) was investigated in this study. For in vitro osteogenic differentiation, both BM-MSCs and C3H10T1/2 cells differentiated to osteoblastic cell lineage and showed positive staining for alkaline phosphatase (ALP) and increased mRNA expression of Runx2, Col1$\alpha$I, and osteocalcin. C3H10T1/2 cells and BM-MSCs induced similar amounts of bone formation in the biomaterials. Under chondrogenic induction in the presence of TGF-$\beta$1, cell pellets of both BM-MSCs and C3H10T1/2 cells formed cartilage-like tissues with cartilage matrix components including proteoglycan, type II collagen, and aggrecan. However, C3H10T1/2 cells presented lower adipogenic differentiation potential, with only about 10% C3H10T1/2 cells (but about 70% of BM-MSCs) being committed to adipogenesis. In this study we confirmed that C3H10T1/2 cells coimplanted with osteoconductive scaffolds can form bone spontaneously in vivo and that C3H10T1/2 cells have a basal level of osteocalcin expression, suggesting that they may be a good alternative source of primary BM-MSCs for investigating osteogenic and chondrogenic differentiation in bone or cartilage tissue engineering studies. Caution is needed when using C3H10T1/2 cells for adipogenic studies as they appear to have lower adipogenic potential than BM-MSCs.

**Keywords** Mesenchymal stem cell · C3H10T1/2 cell · Osteogenesis · Chondrogenesis · Adipogenesis

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Mesenchymal stem cells (MSCs) are pluripotent cells that are capable of self-renewing, and their progeny gives rise to various mesenchymal tissues [1]. Despite their having been isolated from circulating blood [2, 3], cord blood [4, 5], placenta [6], heart [7], amniotic fluid [8], adipose tissue [9], synovium [10], skeletal muscle [11], pancreas [12], and deciduous teeth [13], bone marrow is still considered the most accessible source of MSCs (BM-MSCs). BM-MSCs are capable of differentiating into osteoblasts, chondrocytes, adipocytes, fibroblasts, hepatocytes, neural cells, etc., and can give rise to cartilage [14], bone [15, 16], tendon [17], muscle [18, 19], and many other tissues. BM-MSCs together with biomaterial scaffolds are employed to repair bone defects. Both direct transplantation and application of genetically modified MSCs for the treatment of bone defects and osteogenesis imperfecta have promising therapeutic effects [20, 21]. Other tissues such as cartilage, tendons, ligaments, and even heart are also the targets of MSC therapeutic applications [22–24].

The C3H10T1/2 cell line was established in 1973 from C3H mouse embryos 14–17 days old [25]. Under normal cell culture conditions, C3H10T1/2 cells display fibroblastic morphology, while they are able to develop into osteoblasts, chondrocytes, as well as adipocytes [26–30] under specific inductions. C3H10T1/2 cells acquire osteoblastic phenotype under stimulation of bone morphogenetic proteins (BMPs) [26, 31–33], hedgehog proteins [34], interleukins [35], and Wnt family members [36]. Chondrogenic induction is mostly performed using micromass or monolayer culture systems and under stimulation of BMP-2 [27, 37], TGF-$\beta$1 [29], or a combination of other chondrogenic factors [28]. BMP-2 and BMP-4 could also induce C3H10T1/2 cells to differentiate toward to adipogenic lineage [30, 38–40]. C3H10T1/2 cells have also been used to investigate gene function in vivo [41, 42], but the differentiation capability of C3H10T1/2 cells has not been purposely compared with that of primary BM-MSCs from C3H mice.

BM-MSCs are an attractive cell source for both in vitro study and in vivo tissue repair; however, BM-MSCs show heterogeneity in colony sizes, colony-forming rates, and cell morphology during isolation/expansion [43], and they have spontaneous differentiation potential. In contrast, C3H10T1/2 cells have homogeneous populations and do not undergo spontaneous differentiation under normal culture conditions, and they may be used as an alterative cell source to primary BM-MSCs in many studies. The aim of this study was to compare the differentiation potentials of C3H10T1/2 cells with mouse primary BM-MSCs in culture systems and in transplantation animal experiments.

## Materials and Methods

### Cell Culture and Multilineage Differentiation Induction

Murine C3H10T1/2 cells (ATCC, Manassas, VA, USA) were routinely cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA) with 5% $CO_2$ at 37°C. BM-MSCs of C3H mice (Harlan, Bicester, UK; 3 months old, male) were isolated from femur bone marrow washouts by density gradient centrifugation as previously decribed [16], plated at a density of $2 \times 10^5/cm^2$, and cultured in DMEM containing 10% FBS. For osteogenic induction, both BM-MSCs and C3H10T1/2 cells were cultured in basal medium supplemented with 50 mg/mL L-ascorbic acid, 10 mM $\beta$-glycerophosphate, and $10^{-8}$ M dexamethasone (Sigma, Dorset, UK). For chondrogenic induction, $5 \times 10^5$ cell aliquots were spun down at $500 \times g$ for 5 minutes in 15 mL conical polypropylene tubes and cultured in serum-free medium supplemented with $10^{-7}$ M dexamethasone, 0.2 mM L-ascorbic acid-2 phosphate (Sigma, Dorset, UK), 1 mM sodium pyruvate (BDH Laboratory Supplies, Dorset, UK), BD$^{TM}$ ITS + Premix 1:100, and 10 ng/mL TGF-$\beta$1 (PeproTech EC, London, UK). Medium was changed every 2 days with freshly added TGF-$\beta$1, and the pellets were spun at $500 \times g$ for 5 minutes once a day. For adipogenic induction, cells were cultured in serum-free medium supplemented with 0.5 mM 3-isobutyl-1-methylxanthine, 50 µM indomethacin, and 10 M dexamethasone. The medium was changed every 3 days.

### Cytochemistry Staining

#### *Alkaline Phosphatase (ALP) Staining*

At day 28 of osteogenic induction, cells were stained with the Alkaline Phosphatase Leukocyte kit (Sigma) according to the protocols supplied.

#### *Alcian Blue Staining*

At day 28 of chondrogenic induction, fresh cell pellets were embedded in OCT compound (Tissue-Tek, Hatfield, PA, USA) in cryomolds, snapped in isopentane precooled in liquid nitrogen, and stored at −80°C until needed. Cryostat sections (4–8 µm) were taken and subjected to staining. Sections were fixed in ice-cold acetone for 30 minutes, stained in alcian blue solution (pH 2.5) for 10 minutes, washed well in tap water, then dehydrated and mounted.

#### *Oil Red O Staining*

At 7–10 days of adipogenic induction, cells were washed twice with PBS, fixed with 10% neutral buffered formalin for 30 minutes, stained with 0.5% oil red O in isopropanol:distilled water (60:40) for 30 minutes, then air-dried and photographed under a microscope.

### Immunohistochemical Staining for Type II Collagen on Cryosections

Cryosections derived from chondrogenically induced cell pellets were washed in PBS after fixation in ice-cold acetone, treated with 3% $H_2O_2$ for 5 minutes, and blocked in 10% normal serum and 0.1% bovine serum albumin (BSA) in PBS for 20 minutes. Sections were sequentially incubated with or without type I collagen primary antibody diluted 1:100 for 1 hour, then biotinylated secondary antibody diluted 1:100, then horseradish peroxidase (HRP)–streptavidin, and developed in diaminobenzidine substrate. Sections were counterstained with hematoxylin.

### RNA Isolation and Real-Time PCR

Total RNA was extracted using Trizol (Invitrogen, UK) from C3H10T1/2 cells and C3H BM-MSCs cultured under osteogenic/chondrogenic/adipogenic induction conditions. First-strand cDNA was synthesized from 1 μg of total RNA using the ImProm-II™ Reverse Transcription system (Promega, Southampton, UK). All oligonucleotide PCR primers used in this study are shown in Table 1. mRNA expression levels of target genes were compared using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression in the same RNA preparations as the internal control.

### Nude Mouse Transplantation Experiment

Sterilized Skelite™ porous calcium phosphate resorbable blocks (0.3 × 0.3 × 0.3 cm; Millenium Biologix, Kingston, Canada) were coated with fibronectin (100 mg/mL) prior to cell seeding. C3H10T1/2 cells and BM-MSCs under basal culture conditions were trypsinized with 0.25 M EDTA and 0.05% trypsin (Invitrogen, Abingdon, UK) on the day of implantation and resuspended in PBS at $2.5 \times 10^6$ cells/mL. Cell suspension (0.2 mL, $0.5 \times 10^6$ cells) was loaded onto each block, incubated at 37°C for 3 hours to allow cell attachment, and then delivered to the operating room. Male nude mice (Harlan, UK), 4–6 weeks old, were anesthetized with pentobarbital at a concentration of 50 mg/kg. A 2-cm cut was made alone the middle line at the back, four Skelite blocks with or without cells were implanted into four subcutaneous pockets in each mouse, and the skin cut was sutured. Animals were killed 8 weeks after transplantation.

### Histological Observation

All mice were killed at 8 weeks postsurgery, and the implants were carefully removed. Specimens were fixed with 10% neutral buffered formalin for 48 hours, decalcified in neutral buffered 8% formic acid for 2 weeks, and then embedded in paraffin. Five-micrometer sections were cut and subject to hematoxylin and eosin (H&E) staining. To quantify the amount of bone formation, x400 magnification digital photos were taken at five randomly selected fields in each section and saved in TIFF format. These images were analyzed using image analysis software (Bioquant Nova 4.00.8; Bioquant, Nashville, TN, USA). The average bone tissue in each of the samples was expressed as a percentage pixel of the total area pixel.

## Results

### Comparison of Osteogenic Differentiation between C3H10T1/2 Cells and BM-MSCs

C3H10T1/2 cells were demonstrated to possess similar osteogenic differentiation potential as primary BM-MSCs. After 5–7 days of culture, a small percentage of cells isolated from the mononuclear cells of bone marrow aspirate formed colonies. BM-MSCs were of fibroblastic morphology but larger than C3H10T1/2 cells (Fig. 1a–c). After osteogenic induction for 28 days, in BM-MSCs approximately 40–50% of cells were ALP-positive (Fig. 1d) compared with control cells (Fig. 1e) and there was nodule-like tissue formation; a similar percentage of C3H10T1/2 cells were ALP-positive (Fig. 1f), but there was no nodule-like tissue formation

**Table 1** Nucleotide sequences for PCR primers

| Gene | Gene bank accession number | Size of PCR product (bp) | Primer sequence |
|---|---|---|---|
| *Primers for RT-PCR* | | | |
| Runx2 | NM_009820 | 297 | 5′-GGACGAGGCAAGAGTTTCAC-3′ (F) |
| | | | 5′-TGCCTGCCTGGGATCTGTAA-3′ (R) |
| Col1α1 | NM_007742 | 300 | 5′-CTGACCTTCCTGCGCCTGATGTCC-3′ (F) |
| | | | 5′-GTCTGGGGCACCAACGTCCAAGGG-3′ (R) |
| Osteocalcin | NM_001032298 | 327 | 5′-AGTCACCAACCACAGCATCC-3′ (F) |
| | | | 5′-TTTGTCCCTTCCCTTCTGCC-3′ (R) |
| Col2α1 | NM_031163 | 268 | 5′- GAACAACCAGATCGAGAGCA -3′ (F) |
| | | | 5′- CTCTCCAAACCAGATGTGCT -3′ (R) |
| Aggrecan | NM_007424 | 297 | 5′-CTGGAGACAGGACTGAAATC-3′ (F) |
| | | | 5′-CTCCATTCAGACAAGGGCTT-3′ (R) |
| PPARγ2 | NM_011146 | 550 | 5′-TCTGATTATGGGTGAAACTC-3′(F) |
| | | | 5′-TTTCTACTCTTTTTGTGGATC-3′(R) |

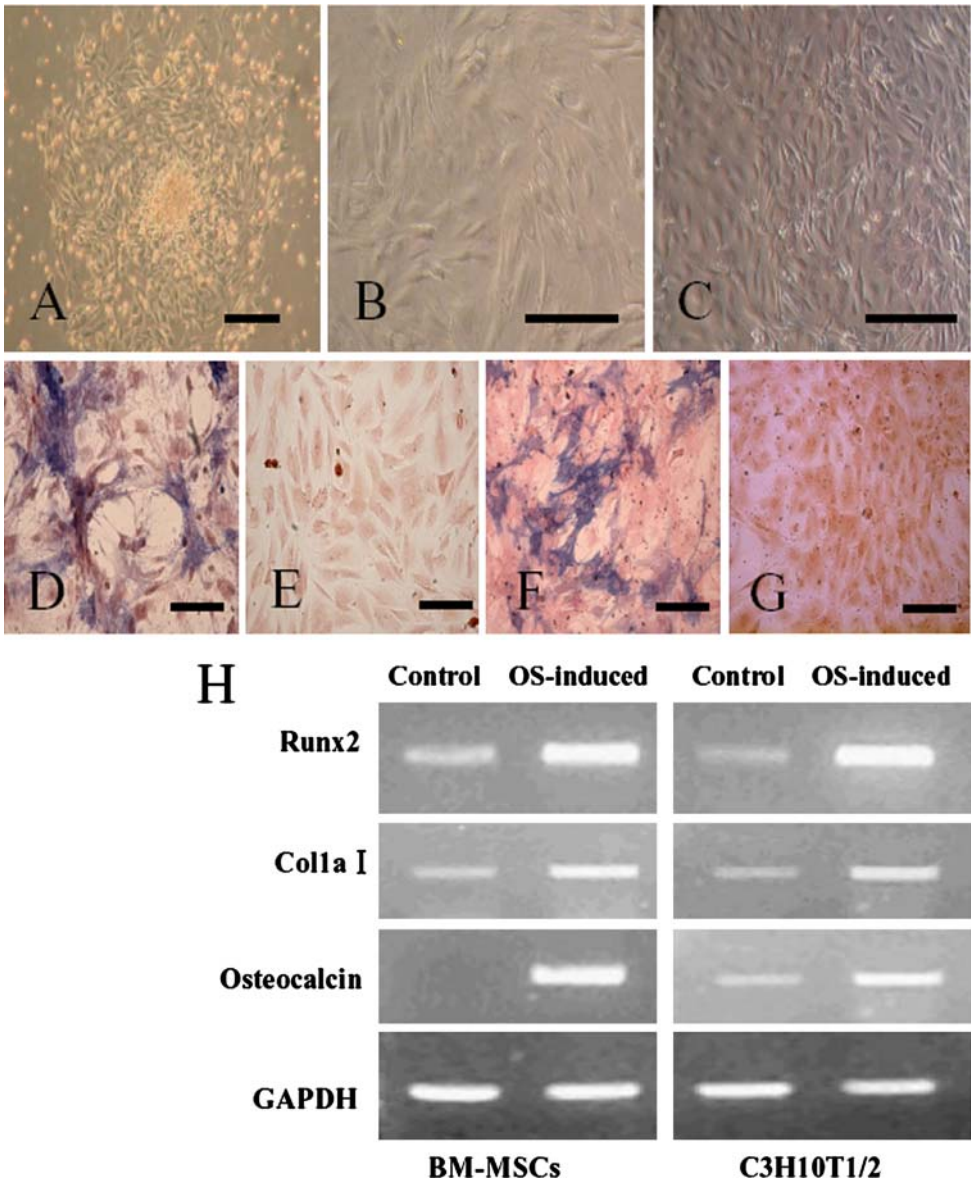


**Fig. 1** Cell culture and osteogenic induction of BM-MSCs and C3H10T1/2 cells. **a** Primary culture and expansion of BM-MSCs. **b** First-passage BM-MSCs in culture. **c** C3H10T1/2 cells in culture. **d** ALP staining in BM-MSCs after osteogenic induction. **e** ALP staining in BM-MSCs without osteogenic induction. **f** ALP staining in C3H10T1/2 cells after osteogenic induction. **g** ALP staining in C3H10T1/2 cells without osteogenic induction. **a–g** Bar = 50 μm. **h** mRNA expression of Runx2, Col1αI, and osteocalcin in BM-MSCs and C3H10T1/2 cells treated with or without osteogenic inductive condition for 28 days, with GAPDH as an internal control

(Fig. 1g). At the mRNA level, BM-MSCs and C3H10T1/2 cells showed analogous expression patterns of osteogenic markers (Fig. 1h). Genes coding transcription factor Runx2 and type I collagen were expressed at the basal level in the two cell types before osteogenic induction, but their expressions were significantly enhanced after osteogenic induction (Fig. 1h). Before osteogenic induction, BM-MSCs had no detectable osteocalcin expression, while C3H10T1/2 cells exhibited a basal level expression of osteocalcin; however, osteocalcin gene expression was significantly enhanced after osteogenic induction in both BM-MSCs and C3H10T1/2 cells. Therefore, C3H10T1/2 cells may possess higher osteogenic differentiation potential in vitro compared to BM-MSCs.

## Comparison of Chondrogenic Differentiation Between C3H10T1/2 Cells and BM-MSCs

C3H10T1/2 cells showed comparable chondrogenic differentiation potential in a pellet induction system as BM-MSCs (Fig. 2). Compared to the control group where TGF-ß1 was absent (Fig. 2 a1–3), BM-MSCs that underwent 4 weeks of chondrogenic pellet induction developed multilayered matrix-rich morphology (Fig. 2 b1–3). C3H10T1/2 cells had similar morphology after 4 weeks of chondrogenic induction (Fig. 2 c1–3). Both cell types showed positive staining for alcian blue (Fig. 2 b2, c2) as well as type II collagen (Fig. 2 b3, c3), confirming the production of cartilage extracellular matrix. RT-PCR results demonstrated that the transcription of genes coding type II

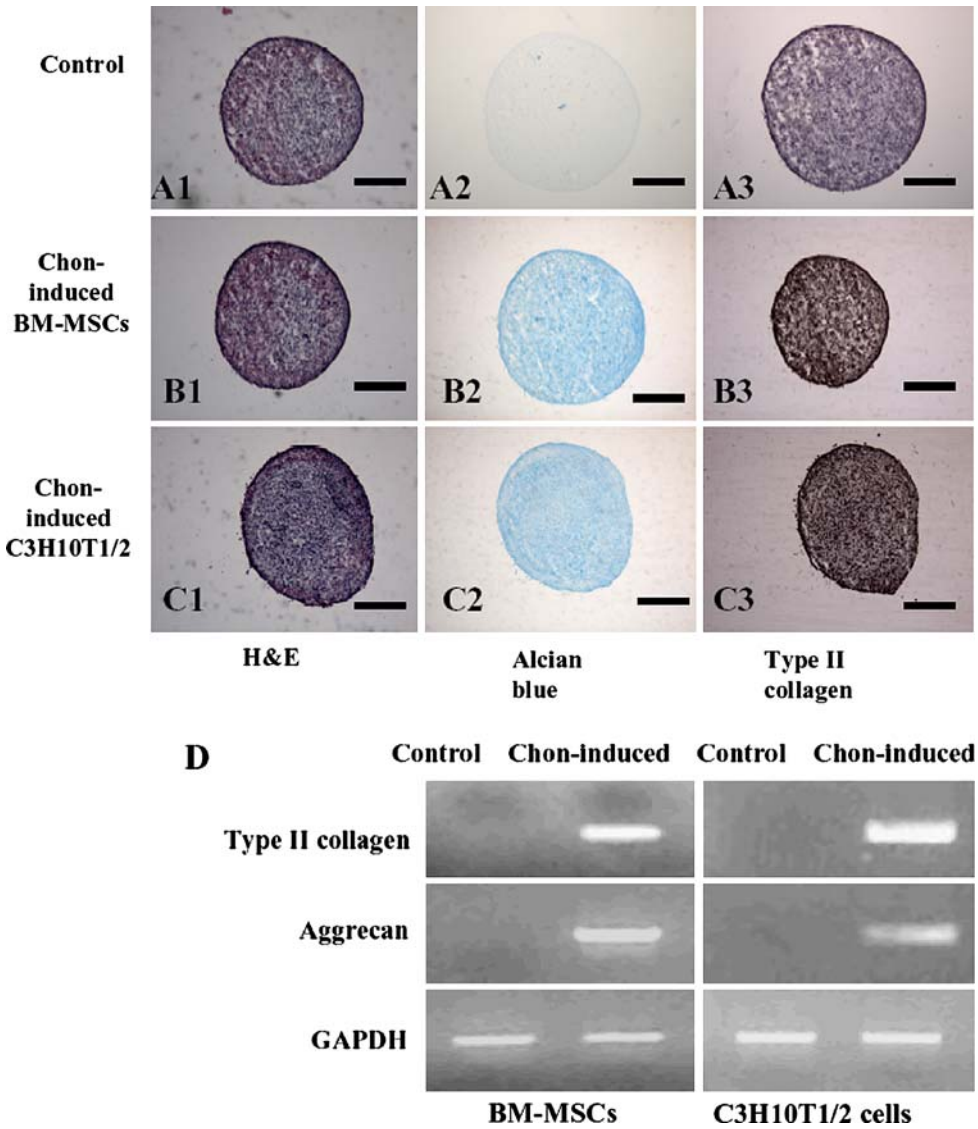


**Fig. 2** Chondrogenic induction of BM-MSCs and C3H10T1/2 cells. **a1–c3** Representative images of H&E staining, alcian blue staining, and immunostaining of type II collagen on BM-MSCs and C3H10T1/2 cells cultured under pellet chondrogenic inductive conditions for 28 days. **a1–c3** Bar = 100 μm. **b** mRNA expression of Col2αI and aggrecan in BM-MSCs and C3H10T1/2 cells under chondrogenic inductive conditions for 28 days, with GAPDH as an internal control

collagen and aggrecan had been induced after chondrogenic induction in both cell types at a comparable level (Fig. 2d).

## Comparison of Adipogenic Differentiation Between C3H10T1/2 Cells and BM-MSCs

Differentiation toward to adipogenic lineage is an important characteristic of BM-MSCs. Culture for 5–10 days under defined conditions led to marked adipogenic phenotype in BM-MSCs, and lipid-rich vacuoles accumulated within approximately 70% of the cells that were positive for oil red O staining (Fig. 3a, b). Only about 10% of C3H10T1/2 cells secreted lipid droplets and stained positively by oil red O (Fig. 3c, d). RT-PCR results showed that peroxisome proliferator-activated receptor γ2 (PPARγ2), a critical transcription factor for adipocyte development, was not detectable in C3H10T1/2 cells and BM-MSCs before adipogenic induction, but it was expressed in both cell types after adipogenic induction (Fig. 3e). mRNA expression of PPARγ2 in BM-MSCs was much stronger than that in C3H10T1/2 cells under adipogenic inductive conditions for 7 days, with GAPDH as an internal control, indicating that C3H10T1/2 cells may possess lower adipogenic differentiation potential compared to BM-MSCs.

## In Vivo Bone Formation of C3H10T1/2 Cells and BM-MSCs

After 8-week implantation in nude mice, bone formation was found in all the implants loaded with either BM-MSCs or C3H10T1/2 cells, but no bone formation was seen in the control group when only scaffolds were implanted (Fig. 4). The newly formed bone was mainly at the porous surfaces inside the scaffolds. We did not see cartilage in either of the groups. There was no significant difference in the amount of

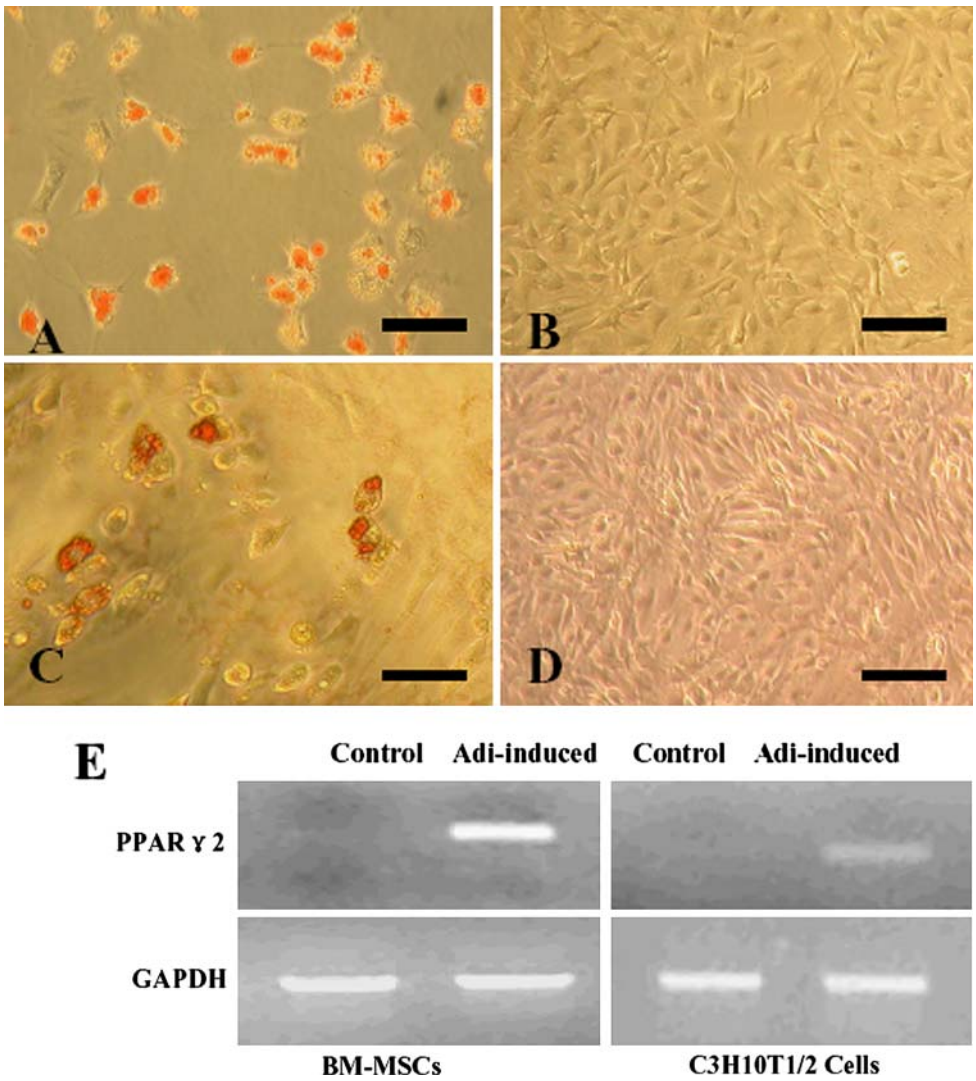


**Fig. 3** Adipogenic differentation of BM-MSCs and C3H10T1/2 cells. Representative images of oil red O staining on BM-MSCs (**a**) and C3H10T1/2 cells (**c**) cultured under adipogenic inductive conditions for 7 days. No positive red O staining is seen in BM-MSCs (**b**) and C3H10T1/2 cells (**d**) cultured without adipogenic induction. **a–d** Bar = 50 μm. **e** mRNA expression of PPARγ2 in BM-MSCs was stronger than that in C3H10T1/2 cells under adipogenic inductive conditions for 7 days, with GAPDH as an internal control

bone formation between BM-MSCs and C3H10T1/2 cells (16.0 ± 5.0 vs. 14.5 ± 7.0%, $P > 0.05$, Student's *t*-test).

## Discussion

MSCs have multipotent differentiation potential and vigorous self-renewal capability. Inducing MSC differentiation to various cell lineages in vitro and in vivo may be used as a model to study the molecular and cellular events during mesenchymal tissue development. Culture-expanded MSCs or genetically modified MSCs have been employed in the regeneration of various tissues, including bone, cartilage, tendons, ligaments, and heart [22–24]. Unselected MSCs contain heterogenous cell populations that may affect their characterization and application. The ratio of MSCs in nucleated marrow cells, determined by colony-forming efficiency, is culture condition–dependent and varies among different species. MSCs also show heterogeneity in colony size, colony-forming rate, as well as morphology, which ranges from fibroblast-like and spindle-shaped cells to large and flat cells [43].

MSCs possess distinct spontaneous differentiation potentials. After culture in basal medium for 20 days, some colonies were strongly positive for ALP while others were negative. Some colonies accumulated fat droplets identified by oil red O staining, whereas others occasionally underwent chondrogenic differentiation and showed positive alcian blue staining [44]. Pittenger et al. [45] reported that only one-third of adherent BM-MSC colonies were pluripotent (osteo-/chondro-/adipogenic), while most colonies exhibited bipotentiality (osteo-/chondrogenic) or unipotentiality (osteogenic). The heterogeneity seen in the MSC differentiation potential is therefore due to mixed cell populations or subpopulations at different differentiation states in the MSC pool [15, 46].

In contrast, C3H10T1/2 cells, an immortalized cell line from mouse embryo, represent a homogeneous cell

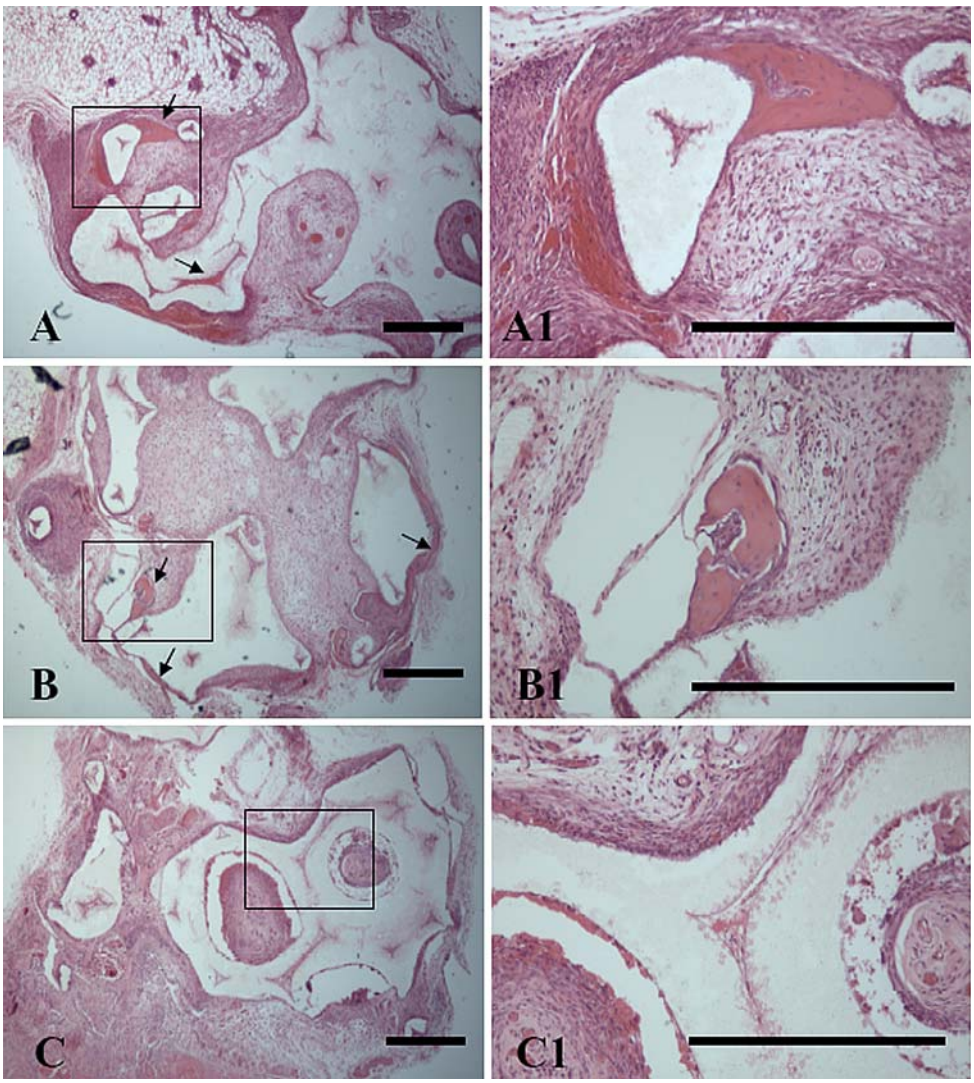


**Fig. 4** Representative H&E staining of the blocks after 8-week implantation in nude mice. **a** Section of block implanted with BM-MSCs for 8 weeks showed new bone formation (*arrows*). **a1** Boxed area in **a**. **b** Section of block implanted with C3H10T1/2 cells for 8 weeks showed new bone formation (*arrows*). **b1** Boxed area in **b**. **c** Section of block loaded with PBS only implanted for 8 weeks showed only fibrous tissue formation. **c1** Boxed area in **c**. All bars = 1 mm

population without spontaneous differentiation under basal culture conditions and yet possess many MSC characteristics. Under the same osteogenic and chondrogenic inductive conditions applied to BM-MSCs, C3H10T1/2 cells showed comparable or similar osteogenic and chondrogenic differentiation potential. However, under the same adipogenic inductive conditions, C3H10T1/2 cells formed a lower percentage of oil red O-positive cells and expressed a lower level of PPAR than primary BM-MSCs. As we have demonstrated that C3H10T1/2 cells have a basal level of osteocalcin gene expression, they may belong to bipotential (osteo/chondro) subpopulations of BM-MSCs. Interestingly, C3H10T1/2 cells could commit to the adipocyte lineage under stimulation of specific growth factors such as BMP-2 and BMP-4 [30, 38–40], suggesting that they may use different signaling pathways for adipogenic differentiation from BM-MSCs.

In conclusion, in this study we have confirmed that C3H10T1/2 cells coimplanted with osteoconductive scaffolds can form bone spontaneously in vivo and that C3H10T1/2 cells have a basal level of osteocalcin expression, suggesting they may be a good alternative cell source of primary BM-MSCs for investigating osteogenic and chondrogenic differentiation in bone or cartilage tissue engineering studies. Caution is needed when using C3H10T1/2 cells for adipogenic studies as they appear to have lower adipogenic potential than BM-MSCs.

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## References

1. Cancedda R, Castagnola P, Cancedda FD, Dozin B, Quarto R (2000) Developmental control of chondrogenesis and osteogenesis. Int J Dev Biol 44:707–714
2. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG (2001) Circulating skeletal stem cells. J Cell Biol 153:1133–1140

3. Luria EA, Panasyuk AF, Friedenstein AY (1971) Fibroblast colony formation from monolayer cultures of blood cells. Transfusion 11:345–349
4. Bieback K, Kern S, Kluter H, Eichler H (2004) Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 22:625–634
5. Romanov YA, Svintsitskaya VA, Smirnov VN (2003) Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells 21:105–110
6. Igura K, Zhang X, Takahashi K, Mitsuru A, Yamaguchi S, Takashi TA (2004) Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. Cytotherapy 6:543–553
7. Warejcka DJ, Harvey R, Taylor BJ, Young HE, Lucas PA (1996) A population of cells isolated from rat heart capable of differentiating into several mesodermal phenotypes. J Surg Res 62:233–242
8. Tsai MS, Lee JL, Chang YJ, Hwang SM (2004) Isolation of human multipotent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. Hum Reprod 19:1450–1456
9. Katz AJ, Tholpady A, Tholpady SS, Shang H, Ogle RC (2005) Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells 23:412–423
10. Fickert S, Fiedler J, Brenner RE (2003) Identification, quantification and isolation of mesenchymal progenitor cells from osteoarthritic synovium by fluorescence automated cell sorting. Osteoarthritis Cartilage 11:790–800
11. Young HE, Mancini ML, Wright RP, Smith JC, Black AC Jr, Reagan CR, Lucas PA (1995) Mesenchymal stem cells reside within the connective tissues of many organs. Dev Dyn 202: 137–144
12. Hu Y, Liao L, Wang Q, Ma L, Ma G, Jiang X, Zhao RC (2003) Isolation and identification of mesenchymal stem cells from human fetal pancreas. J Lab Clin Med 141:342–349
13. Qu Q, Harkonen PL, Vaananen HK (1999) Comparative effects of estrogen and antiestrogens on differentiation of osteoblasts in mouse bone marrow culture. J Cell Biochem 73:500–507
14. Kadiyala S, Young RG, Thiede MA, Bruder SP (1997) Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant 6: 125–134
15. Bruder SP, Jaiswal N, Haynesworth SE (1997) Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem 64:278–294
16. Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, Kadiyala S (1998) Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. J Orthop Res 16: 155–162
17. Young RG, Butler DL, Weber W, Caplan AI, Gordon SL, Fink DJ (1998) Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res 16:406–413
18. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279: 1528–1530
19. Galmiche MC, Koteliansky VE, Briere J, Herve P, Charbord P (1993) Stromal cells from human long-term marrow cultures are mesenchymal cells that differentiate following a vascular smooth muscle differentiation pathway. Blood 82:66–76
20. Chamberlain JR, Schwarze U, Wang PR, Hirata RK, Hankenson KD, Pace JM, Underwood RA, Song KM, Sussman M, Byers PH, Russell DW (2004) Gene targeting in stem cells from individuals with osteogenesis imperfecta. Science 303:1198–1201
21. Le Blanc K, Gotherstrom C, Ringden O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Norden-Lindeberg S, Jansson M, Dalton A, Astrom E, Westgren M (2005) Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. Transplantation 79:1607–1614
22. Hui JH, Ouyang HW, Hutmacher DW, Goh JC, Lee EH (2005) Mesenchymal stem cells in musculoskeletal tissue engineering: a review of recent advances in National University of Singapore. Ann Acad Med Singapore 34:206–212
23. Ichinose S, Yamagata K, Sekiya I, Muneta T, Tagami M (2005) Detailed examination of cartilage formation and endochondral ossification using human mesenchymal stem cells. Clin Exp Pharmacol Physiol 32:561–570
24. Young HE, Ceballos EM, Smith JC, Mancini ML, Wright RP, Ragan BL, Bushell I, Lucas PA (1993) Pluripotent mesenchymal stem cells reside within avian connective tissue matrices. In Vitro Cell Dev Biol Anim 29A:723–736
25. Reznikoff CA, Brankow DW, Heidelberger C (1973) Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res 33:3231–3238
26. Katagiri T, Yamaguchi A, Ikeda T, Yoshiki S, Wozney JM, Rosen V, Wang EA, Tanaka H, Omura S, Suda T (1990) The non-osteogenic mouse pluripotent cell line, C3H10T1/2, is induced to differentiate into osteoblastic cells by recombinant human bone morphogenetic protein-2. Biochem Biophys Res Commun 172:295–299
27. Denker AE, Haas AR, Nicoll SB, Tuan RS (1999) Chondrogenic differentiation of murine C3H10T1/2 multipotential mesenchymal cells: I. Stimulation by bone morphogenetic protein-2 in high-density micromass cultures. Differentiation 64:67–76
28. Atkinson BL, Fantle KS, Benedict JJ, Huffer WE, Gutierrez-Hartmann A (1997) Combination of osteoinductive bone proteins differentiates mesenchymal C3H/10T1/2 cells specifically to the cartilage lineage. J Cell Biochem 65:325–339
29. Denker AE, Nicoll SB, Tuan RS (1995) Formation of cartilage-like spheroids by micromass cultures of murine C3H10T1/2 cells upon treatment with transforming growth factor-beta 1. Differentiation 59:25–34
30. Tang QQ, Otto TC, Lane MD (2004) Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc Natl Acad Sci USA 101:9607–9611
31. Shea CM, Edgar CM, Einhorn TA, Gerstenfeld LC (2003) BMP treatment of C3H10T1/2 mesenchymal stem cells induces both chondrogenesis and osteogenesis. J Cell Biochem 90:1112–1127
32. Date T, Doiguchi Y, Nobuta M, Shindo H (2004) Bone morphogenetic protein-2 induces differentiation of multipotent C3H10T1/2 cells into osteoblasts, chondrocytes, and adipocytes in vivo and in vitro. J Orthop Sci 9:503–508
33. Spinella-Jaegle S, Roman-Roman S, Faucheu C, Dunn FW, Kawai S, Gallea S, Stiot V, Blanchet AM, Courtois B, Baron R, Rawadi G (2001) Opposite effects of bone morphogenetic protein-2 and transforming growth factor-beta1 on osteoblast differentiation. Bone 29:323–330
34. Nakamura T, Aikawa T, Iwamoto-Enomoto M, Iwamoto M, Higuchi Y, Pacifici M, Kinto N, Yamaguchi A, Noji S, Kurisu K, Matsuya T (1997) Induction of osteogenic differentiation by hedgehog proteins. Biochem Biophys Res Commun 237:465–469
35. Suga K, Saitoh M, Fukushima S, Takahashi K, Nara H, Yasuda S, Miyata K (2001) Interleukin-11 induces osteoblast differentiation and acts synergistically with bone morphogenetic protein-2 in C3H10T1/2 cells. J Interferon Cytokine Res 21:695–707

36. Derfoul A, Carlberg AL, Tuan RS, Hall DJ (2004) Differential regulation of osteogenic marker gene expression by Wnt-3a in embryonic mesenchymal multipotential progenitor cells. Differentiation 72:209–223
37. Zehentner BK, Dony C, Burtscher H (1999) The transcription factor Sox9 is involved in BMP-2 signaling. J Bone Miner Res 14:1734–1741
38. Mie M, Ohgushi H, Yanagida Y, Haruyama T, Kobatake E, Aizawa M (2000) Osteogenesis coordinated in C3H10T1/2 cells by adipogenesis-dependent BMP-2 expression system. Tissue Eng 6:9–18
39. Zehentner BK, Leser U, Burtscher H (2000) BMP-2 and sonic hedgehog have contrary effects on adipocyte-like differentiation of C3H10T1/2 cells. DNA Cell Biol 19:275–281
40. Hata K, Nishimura R, Ikeda F, Yamashita K, Matsubara T, Nokubi T, Yoneda T (2003) Differential roles of Smad1 and p38 kinase in regulation of peroxisome proliferator-activating receptor gamma during bone morphogenetic protein 2-induced adipogenesis. Mol Biol Cell 14:545–555
41. Hoffmann A, Czichos S, Kaps C, Bachner D, Mayer H, Kurkalli BG, Zilberman Y, Turgeman G, Pelled G, Gross G, Gazit D (2002) The T-box transcription factor Brachyury mediates cartilage development in mesenchymal stem cell line C3H10T1/2. J Cell Sci 115:769–781
42. Noel D, Gazit D, Bouquet C, Apparailly F, Bony C, Plence P, Millet V, Turgeman G, Perricaudet M, Sany J, Jorgensen C (2004) Short-term BMP-2 expression is sufficient for in vivo osteochondral differentiation of mesenchymal stem cells. Stem Cells 22:74–85
43. Bianco P, Riminucci M, Gronthos S, Robey PG (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 19:180–192
44. Herbertson A, Aubin JE (1997) Cell sorting enriches osteogenic populations in rat bone marrow stromal cell cultures. Bone 21:491–500
45. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
46. Baksh D, Song L, Tuan RS (2004) Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med 8:301–316