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ORIGINAL ARTICLE

In-vitro differentiation study on isolated human mesenchymal stem cells

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Abstract

Mesenchymal stem cells are pluripotent progenitors that could be found in human bone marrow. Mesenchymal stem cells are capable of renewing themselves without differentiation in long-term culture. These cells also have low immunogenicity and can suppress alloreactive T cell responses. In the current study, mesenchymal stem cells isolated and propagated previously from the bone marrow of a megaloblastic anaemia patient were tested for their capabilities to differentiate into adipocytes, chondrocytes and osteoblasts *in vitro*. The differentiated cells were determined by Oil Red O, Alcian Blue-PAS and Alizarin Red S staining, and reverse transcriptase-polymerase chain reaction to determine the expression of mRNA specific for adipogenesis, chondrogenesis and osteogenesis. The results showed that the fibroblast-like cells were capable of differentiating into adipocytes, chondrocytes and osteoblasts upon chemical induction. The adipocytes, chondrocytes and osteoblasts were stained positively to Oil Red O, Alcian Blue-PAS and Alizarin Red S respectively. The differentiated cells were also found to express mRNA specific for adipogenesis ('*peroxisome proliferation-activated receptor γ2*' and lipoprotein lipase), chondrogenesis (collagen type II) and osteogenesis (osteocalcin, osteopontin and alkaline phosphatase). In conclusion, this research has successfully isolated fibroblast-like cells from human bone marrow and these cells demonstrated morphological, cytochemical and immunochemical characteristics similar to mesenchymal stem cells. These cells maintain their proliferative properties and could be differentiated into the mesoderm lineage. The success of this study is vital because mesenchymal stem cells can be used in cellular therapy to regenerate or replace damaged tissues, or as a vehicle for therapeutic gene delivery in the future.

Key words: Mesenchymal stem cells, bone marrow, adipocytes, chondrocytes, osteoblasts

INTRODUCTION

When Prometheus transgressed the law of the ancient gods and stole fire for humankind, to teach them civilization and the arts, his punishment was typically brutal. Jupiter had the great Titan chained to the side of Mount Caucasus, where a vulture preyed daily on his liver, which was renewed as quickly as it was devoured. We mere mortals do not possess livers with quite so vigorous a regenerative capacity, but the legend captures well the remarkable potential of the body to rebuild itself. Throughout our lives we sustain less gruesome injuries from which we recover spontaneously, often without realizing we were hurt. Wound healing involves the recruitment and proliferation of stem cells capable of restoring tissues and even organs to their original form and function.[1]

Bone marrow contains at least two types of stem cells, i.e. hematopoietic stem cells and mesenchymal stem cells (MSC). MSC were first discovered from the human bone marrow by Friedenstein in 1974 as long spindle-shaped cells, adhering on plastic culture dishes. His work was forgotten until recently.[2,3] The capability to also differentiate into the ectoderm (neuron), endoderm (hepatocyte) and mesoderm lineages (cardiomyocyte)[4-7] *in vitro* and *in vivo* suggests that MSC might have pluripotency similar to that

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of embryonic stem cells (ESC). Manifestation of the expression of ESC genetic markers, e.g. Oct-4, Rex-1 and SSEA-1 has again suggested that MSC could be the remnant of ESC that exists throughout the life of adults.[8] If true, MSC could trigger a revolution in medical research as they can be used to replace ESC for studies in differentiation, cellular and gene therapy.

We have previously isolated and characterized MSC,[9] and would like to test the capability of MSC to differentiate into adipocytes, chondrocytes and osteoblasts in this study.

MATERIALS AND METHODS

Isolation and identification of bone marrow mesenchymal stem cells

To isolate human MSC, a bone marrow aspirate was taken from the iliac crest of a megaloblastic anaemia patient who came for routine check up in Hospital Universiti Kebangsaan Malaysia after informed consent and under a protocol approved by the UKM Research Committee and Ethics Committee. Five ml of bone marrow aspirate was layered on top of 3 ml Ficoll-Pague (Amersham Biosciences; Uppsala, Sweden) and centrifuged at 400 g for 30 minutes. The mononuclear cells in the interface (density gradient 1.077 g/l) were extracted and washed twice with culture medium by centrifuging at 100 g for 10 minutes. The pellet of cells were then suspended in Dulbecco's Modified Eagle Medium (DMEM) (Gibco; Grand Island, New York, USA) and the viability of cells was counted by haematocytometer and Trypan Blue staining. Our result showed that the percentage of viable cells was 99.7 %. The cells were then seeded at a density of 1 X 10^7 cells in a 25 cm^2 plastic flask containing DMEM supplemented with 10.0 % Fetal Bovine Serum (FBS) (Gibco; Grand Island, New York, USA). The flask was then incubated in 5 % CO2 in air and monitored daily. Once the cells reached confluency, they were detached by 1 ml of 0.25% Trypsin-EDTA (Gibco; Grand Island, New York, USA) and replated again into new flasks at a similar cell density. Characterization of DMEM-derived adherent cells was performed by using cells from the third and fourth passages after four to five weeks from the initial culture. These cells were stained cytochemically and immunophenotyped as described previously to document their cytochemical and immunochemical properties.[9]

Differentiation study

Differentiation into adipocytes

To induce adipogenesis, adipogenic induction medium was prepared with DMEM supplemented with 1.0 µM dexamethasone, 0.2 mM indomethacin, 0.01 mg/mL insulin, 0.5 mM 3-isobutyl-1-methyl-xanthine, 10.0% FBS, 0.05 U/mL penicillin and 0.05 µg/mL streptomycin. MSC was plated at a density of 4.0 X10^4 cells per cm^2 of plastic culture flasks and incubated in a humidified atmosphere at 37°C with 5% CO_2. After confluency was achieved, adipogenic induction medium was placed on the cells and the induction medium was changed every three days continuously for two to three weeks. Oil Red O was used as a histological stain to visualize the presence of lipid droplets. Reverse transcription-polymerase chain reaction (RT-PCR) was used to analyze the expression of adipogenesis-specific genes.

Differentiation into chondrocytes

To induce chondrogenesis, 3-dimensional cell cultures were maintained in a chemically defined basal medium consisting of DMEM supplemented with 50 µg/ml ascorbate-2-phosphate, 1.0 mM sodium piruvate, 40.0 µg/ml proline, 10.0 ng/ml transforming growth factor-β3, 6.25 µg/ml human insulin, 6.25 µg/ml transferrin, 6.25 µg/ml bovine insulin, 6.25 µg/ml selenous acid, 1.25 µg/ml linoleic acid, 5.35 µg/ml bovine serum albumin, 0.05 U/mL penicillin and 0.05 µg/mL streptomycin.

The 3-dimensional chondrogenic system utilized 1.0 X 10^6 MSC per pellet. MSC were suspended in 1 ml of chondrogenic medium. Cells were centrifuged in 15 ml conical tubes at 200 g for 10 minutes at room temperature to create a pellet. After centrifugation, the caps on the conical tubes were loosened for gas exchange and the pellets were incubated in a humidified atmosphere at 37 °C with 5 % CO2, with medium changes every three to four days. After every medium change, the pellet was agitated to ensure that the pellet was free floating and spheroid. Chondrogenic pellets were harvested after five weeks in culture. To assess chondrogenesis, Alcian Blue-PAS was used to stain cartilage matrix, and RT-PCR was used to analyze chondrogenesis-specific genes.

Differentiation into osteoblasts

To induce osteogenesis, osteogenic induction medium was prepared by supplementing

DMEM with 10% FBS, 50.0 μg/mL ascorbate-2-phosphate, 10.0 mM β-glycerophosphate, 100 nM dexamethasone, 0.05 U/mL penicillin and 0.05 μg/mL streptomycin. MSC were plated at a density of 2.4 $X10^4$ cells per cm^2 of plastic culture flasks and incubated in osteogenic induction medium in a humidified atmosphere at 37°C with 5% CO_2. The medium was changed every three days continously for two to three weeks. Alizarin Red S was used to stain matrix mineralization associated with osteoblasts, and RT-PCR was used to analyze expression of osteogenesis-specific genes.

Reverse transcriptase-polymerase chain reaction
Total RNA from cells was isolated using TriReagent (Molecular Research Center; Cincinnati, Ohio, Canada) according to manufacturer's protocol. RT-PCR was carried out using BcaBest RNA PCR Kit (Takara Bio Inc., Otsu, Shiga, Japan) according to manufacturer's protocol. To ensure equal distribution of target, a master mix containing all components except specific primers (Table 1) was generated and then aliquoted to each reaction tube. PCR were performed following 30 cycles of amplification (94°C, 15 seconds; 58°C, 45 seconds; and 72°C, 30 seconds) using Eppendorf Mastercycler Gradient and then the PCR reaction was resolved on a 1.2% agarose gel. The band was observed under UV light and photographed.

RESULTS

Isolation and characterization of MSC from human bone marrow aspirates
Isolated MNC from the bone marrow adhered as fibroblast-like cells (Fig. 1). These cells propagated rapidly and when cells at passage 3 were immunophenotyped, they showed abundant CD13 and CD56, and moderate CD54 expression. However, the cells did not have the expression of CD3, CD11c, CD14, CD34, CD45 and HLA-DR, eliminating the presence of contaminating hematopoietic cells. These cells were also positive to NSE and PAS, but not to NASDA, SBB and ALP stains as reported previously.[9]

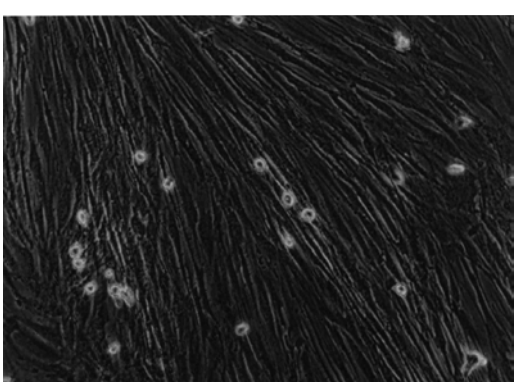


FIG. 1: Fibroblast-like cells colonizing the surface of plastic cell culture flask (x400).

Differentiation into adipocytes
After three to five days of incubation in adipogenic medium, fibroblast-like cells were observed to change into large polygonal cells. Small refractile vesicles believed to be lipids were seen in the cytoplasms and these vesicles become enlarged and fused together to form bigger lipid droplets. At the end of incubation period, almost 90% of the confluent cells had lipid droplets. The lipid was found to stain positively with Oil Red O (Fig. 2A). The RT-PCR results showed that the mRNA of peroxisome proliferation-activated receptor γ2 (PPAR-γ2) (Fig. 3A, Lane 7) and lipoprotein lipase (LPL) (Fig. 3B, Lane 5) were expressed by the MSC after the incubation with adipogenic medium, whereas un-induced cells did not express both adipogenesis specific mRNA (Fig. 3A, Lane 3 and Fig. 3B, Lane 3). A positive control using liposuction tissues was carried out and it showed a positive band of PPAR-γ2 (Fig. 3A, Lane 5) and LPL (Fig. 3B, Lane 7).

Differentiation into chondrocytes
After four to five weeks of incubation in chondrogenic medium, the pellet was fixated and stained with Alcian Blue-PAS. Chondrocytes were seen occupying the lacunars and they were separated from each other by the matrix. Some of the lacunars were found to contain more than two chondrocytes known as isogenous group. When observed under high magnification, the territorial matrix containing high negatively charged glycosaminoglycans was stained blue with Alcian Blue, whereas the interterritorial matrix containing low negatively charged glycosaminoglycans and collagen was stained pink with PAS (Fig. 2B). RT-PCR result showed that the chondrogenic pellet expressed collagen type II whereas un-induced MSC did not express the respective mRNA (Fig. 4, Lane 5). A positive control using human cartilage sample showed a positive band of collagen type II (Fig. 4, Lane 7).

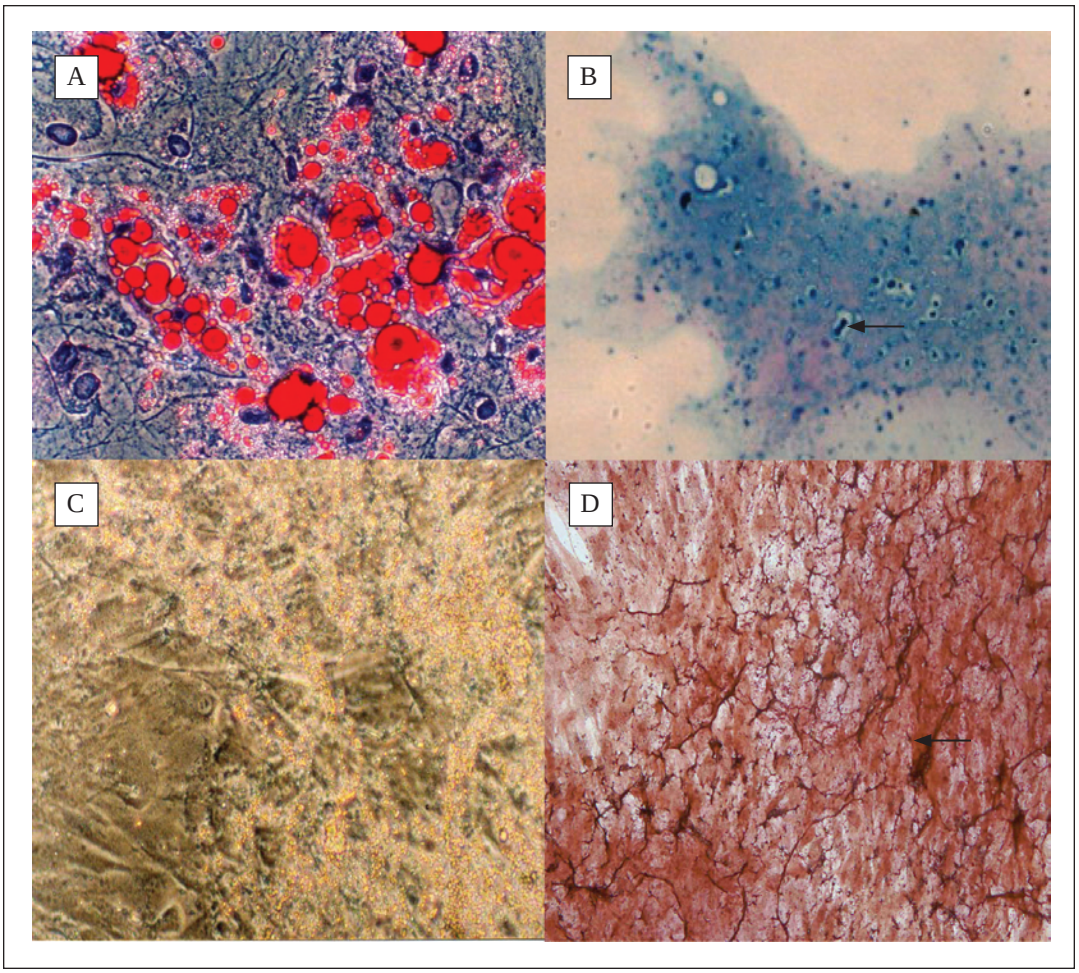


FIG. 2 Differentiation into adipocytes, chondrocytes and osteoblasts. (**A**) Oil Red O staining on adipogenic-induced MSC. Small lipid droplets were present on the cytoplasm of the cells during early incubation and they became large due to lipid accumulation. The lipids were stained red with Oil Red O whereas the nucleus were stained blue with haematoxylin. (**B**) Alcian Blue-PAS stain of chondrogenic-induced MSC. Chondrocytes were seen occupying the lacunars and was surrounded by territorial matrix which were stained blue. The interterritorial matrix was stained pink. Some lacunars contained two chondrocytes to form isogenous group (→*). (**C**) Morphological observation of osteogenic induced MSC. Crystals were seen deposited on most of the surface of monolayer cells at the end of incubation period. The over-crowded crystals made the morphologies of the cells difficult to be distinguished (x400). (**D**) The crystals suspected to be hydroxyapatite formed blue-black lake with sharp edges (→) when stained with hematoxylin. Meanwhile, the amorphous calcium phosphate formed by the action of alkaline phosphatase on matrix was stained orange red at pH4.0 with Alizarin Red S. Both alkaline phosphate and matrix were produced by osteoblasts (x400).

Differentiation into osteogenic lineage cells

After four days of incubation in the osteogenic medium, the fibroblast-like cells started to divide rapidly and become confluent in the plastic culture flasks. Some crystals were seen deposited sparsely on the cells. The number of crystals increased and became very crowded towards the end of the incubation, and this had caused difficulty in identifying the morphology of the cells (Fig. 2C). The whole monolayer cells appeared orange-red when stained with Alizarin Red S at pH 4.0 and some black-blue lake with sharp edges were observed too when the culture was counterstained with Haematoxylin (Fig. 2D). In the meantime, the un-induced MSC culture proliferated much slower than the osteogenic-

TABLE 1: Sequences of RT-PCR primers for analysis of differentiation specific gene expression

Gene		Primer sequences	Size (bp)
Adipose specific genes			
PPARγ2	Sense	5'- GCT GTT ATG GGT GAA ACT CTG – 3'	351
	Antisense	5' – ATA AGG TGG AGA TGC AGG CTC – 3'	
LPL	Sense	5'- GAG ATT TCT CTG TAT GGC ACC –3'	276
	Antisense	5'- CTG CAA ATG AGA CAC TTT CTC –3'	
Cartilage specific gene			
Type II Collagen	Sense	5′- CTG GCA AAG ATG GTG AGA CAG GTG -3′	294
	Antisense	5′- GAC CAT CAG TGC CAG GAG TGC -3′	
Bone specific genes			
Osteocalcin	Sense	5'- ATG AGA GCC CTC ACA CTC CTC –3'	294
	Antisense	5'- GCC GTA GAA GCG CCG ATA GGC –3'	
Osteopontin	Sense	5' – CAC CTG TGC CAT ACC AGT TAA AC –3'	220
	Antisense	5' – ATC CAT GTG GTC ATG GCT TT –3'	
ALP	Sense	5' – GTA CTG GCG AGA CCA AGC G –3'	200
	Antisense	5' – AGG GGA ACT TGT CCA TCT CC –3'	
Endogenous control for cDNA			
β-actin	Sense	5'- GGC ACC CAG CAC AAT GAA GA –3'	629
	Antisense	5'- GGC ACG AAG GCT CAT CAT TC –3'	

Reverse transcription profile of adipogenic-induced MSC

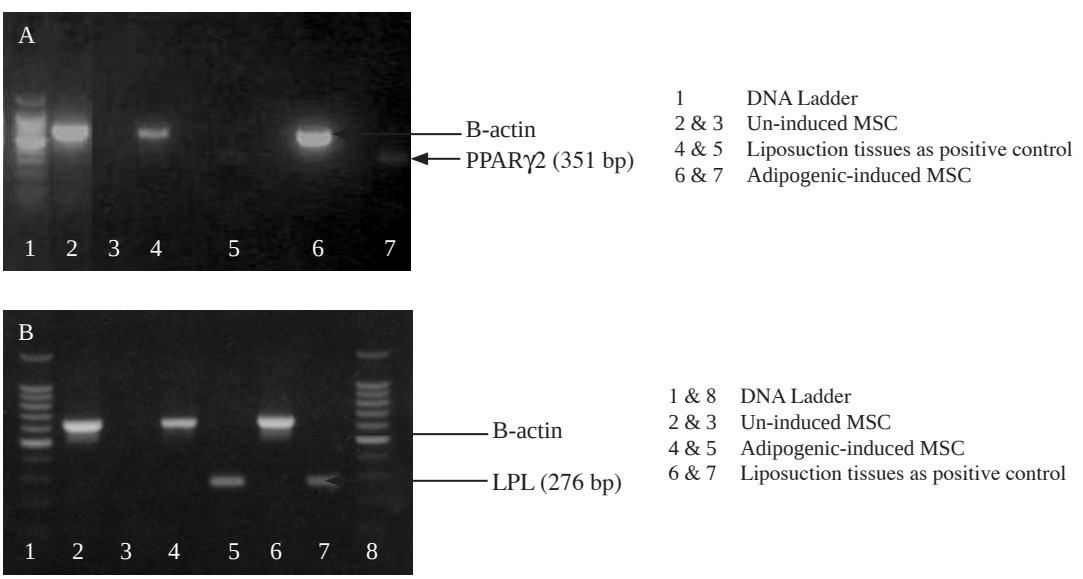


FIG. 3: Reverse transcription profile of adipogenic-induced DMEM-derived adherent cells. The mRNA specific genes for adipogenesis being examined were peroxisome proliferation-activated receptor γ2 (PPAR-γ2) (**A**), and lipoprotein lipase (LPL) (**B**). β-actin was used as a housekeeping gene.

induced culture and no crystals were seen. The un-induced cells stained negatively with Alizarin Red S. The RT-PCR results showed that both induced and un-induced cells had alkaline phosphatase, osteocalcin and osteopontin. However, the induced cells expressed higher alkaline phosphatase (Fig. 5A, Lane 2) and lower osteocalcin (Fig. 5B, Lane 5) and osteopontin (Fig. 5C, Lane 2) compared with that of un-induced cells (Fig. 5A, Lane 3; Fig. 5B, Lane 3; Fig. 5C, Lane 3). To verify that both induced and un-induced cells showed true positive results, bone chips and fibroblasts were used as positive (Fig. 5B, Lane 7) and negative controls (Fig. 5B, Lane 9) in the osteocalcin mRNA expression study. However, only bone chips were used as positive control in alkaline phosphatase (Fig. 5A, Lane 4) and osteopontin (Fig. 5C, Lane 4) mRNA expression studies.

DISCUSSION

We have previously isolated and identified fibroblast-like cells from the bone marrow of a non-malignant blood disorder patient.[9] These cells showed morphological, cytochemical and immunochemical characteristics with resemblance to mesenchymal stem cells. In this study, isolated MSC were successfully induced into the adipocytes, chondrocytes and osteoblasts which belong to the mesoderm lineage confirming that isolated MSC showed properties of stem cells.

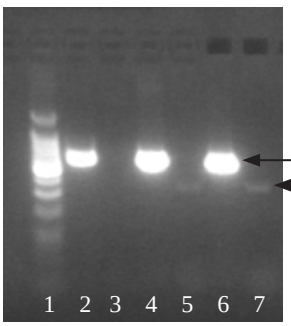
Differentiation into adipocytes was confirmed by the presence of lipid droplets in the induced cells as shown by Oil Red O stain (Fig. 2A). Besides cytochemical staining, adipocytes could also be confirmed by determining the presence of a set of biochemical markers that were involved in changing fatty acids into triglycerides like the stearoyl-coA-desaturase (SCDI) or insulin responsive glucose transporter (GLUT4) gene. Besides that, the CCAT enhancer binding protein (C/EBP) is also reported to exist much earlier than the rest of the adipogenic genes, and thus is believed to be involved in the development of the adipocytes. Other markers which are of less importance include PPARγ2 and LPL. The PPARγ2 is important in initiating the transcription of adipogenic genes and this protein can be activated by fatty acids. In the lipoprotein metabolism, LPL breaks the big molecule of triglycerides into chylomicron and very light density lipoprotein (VLDL) into small molecule of fatty acids to facilitate absorption into tissues through the endothelium of the capillary. Synthesis of LPL is found to be dominant in adipose tissue, cardiac and skeletal muscles, and lactating mammary gland. The synthesized LPL will be translocated into the surface of endothelium cells of the capillary to facilitate the hydrolysis of the lipoprotein.[10,11]

In the RT-PCR study, we found that cells incubated in the adipogenic medium exhibited positive expression of PPARγ2 and LPL, whereas cells without induction exhibited no expression of these genes (Fig. 3). From both the staining and molecular studies, we conclude that the adipogenic medium has successfully induced the differentiation of the MSC into adipocytes.

While adipogenic and osteogenic differentiation studies were performed on monolayer cell cultures, the chondrogenic culture was conducted in the form of a pellet. This is because chondrocytes isolated from human can experience dedifferentiation when cultured on monolayer, marked by morphological changes (from round shape into fibroblast-like cells) and decrease of collagen and cartilage protein synthesis. However, these cells will return to their chondrocytic phenotypes when put into the pellet culture again. This implies that chondrogenic induction demands a very close contact between the progenitor or precursor cells.[12]

Although morphological changes could not be observed during culture in this study, chondrogenesis could still be assessed via Alcian-Blue PAS and expression of collagen type II. Histological staining showed the presence of single chondrocyte settling in unstained cavities believed to be lacunars (Fig. 2B). There were also lacunars filled with two chondrocytes forming isogenous groups. The chondrocytes were embedded in extracellular matrix. The periphery of the lacunars stained with Alcian Blue was believed to be the territorial matrix whereas areas stained pink with PAS were interterritorial matrix. The territorial matrix was rich with chondroitin sulfate (a negatively charged glycosaminoglycan) and contained less collagen type II, whereas the interterritorial matrix was rich with collagen type II and contained less chondroitin sulfate. The Alcian Blue is more basophilic compared with PAS stain, and thus, it stained the matrix territorial. The collagen type II has the same refractive index as the matrix and thus appears invisible under the light microscope. Chondrocytes were oval and located densely at the periphery of the pellet, whereas chondrocytes sitting further from the periphery were bigger and rounder, and fewer in number.[13]

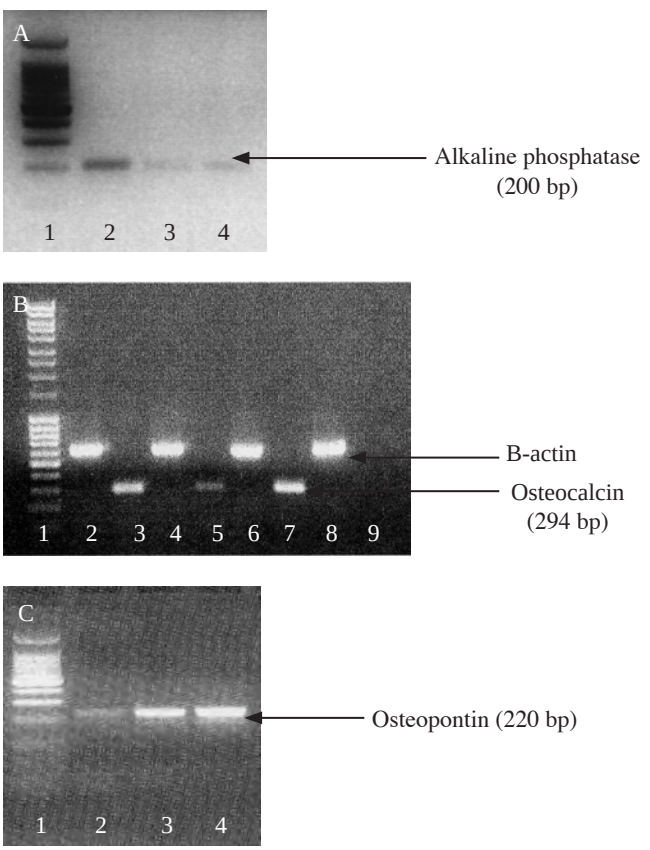
Reverse transcription profile of chondrogenic-induced MSC



1	DNA Ladder
2 & 3	Un-induced MSC
4 & 5	Chondrogenic-induced MSC
6 & 7	Cartilage as positive control

FIG. 4: Collagen type II mRNA expression by the un-induced and chondrogenic-induced DMEM-derived adherent cells. β-actin was used as a housekeeping gene.

Reverse transcription profile of osteogenic-induced MSC



1	DNA Ladder
2	Osteogenic-induced MSC
3	Un-induced MSC
4	Bone chips as positive control

1	DNA Ladder
2 & 3	Un-induced MSC
4 & 5	Osteogenic-induced MSC
6 & 7	Bone chips as positive control
8 & 9	Fibroblasts as negative control

1	DNA Ladder
2	Osteogenic-induced MSC
3	Un-induced MSC
4	Bone chips as positive control

FIG. 5: Reverse transcription profile of osteogenic-induced DMEM-derived adherent cells. The mRNA specific genes for osteogenesis being examined were osteocalcin (**A**), osteopontin (**B**), and alkaline phosphatase (**C**). β-actin was used as a housekeeping gene.

The RT-PCR result showed the MSC pellet incubated in the chondrogenic medium showed expression of collagen type II mRNA compared with un-induced MSC pellet (Fig. 4, Lane 5). The collagen type II gene was chosen for determination of chondrogenesis because the collagen type II constitutes 40% of the total dry weight of the cartilage and 90% of the collagen being synthesized and secreted from the chondrocytes into the matrix in the hyaline and elastic cartilage is collagen type II. Other expression of novel markers for chondrogenesis like aggrecan, collagen type IX and chondroitin sulfate were however not determined in this study.[14-16]

In the osteogenic differentiation, MSC incubated in the osteogenic medium were found to proliferate very rapidly compared with the un-induced MSC. Induced MSC showed positive staining to Alizarin Red S at pH 4.0 (Fig. 2D). The orange-red stain in the monolayer cells was believed to be calcium phosphate amorphous which was formed by the reaction of the alkaline phosphatase (secreted by the osteoblasts) on to the matrix. Meanwhile the blue-black lakes with edges were believed to be calcium crystals presenting themselves in the form of hydroxyapatite.[17]

The results of RT-PCR showed that the osteocalcin, osteopontin and alkaline phosphatase were not good markers for osteogenesis as the un-induced cells (P7) had also expressed these mRNAs (Fig. 5A, Lane 3; Fig. 5B, Lane 3; Fig. 5C, Lane 3). Alkaline phosphatase cytochemical staining using earlier cell culture were not positive (not shown) and thus the expression of alkaline phosphatase might be induced by serum during long term culture. The serum might contain certain agents (e.g. platelet derived growth factor, fibroblasts growth factor and etc.) that induced the proliferation of MSC and differentiation into pre-osteoblasts. Further differentiation into osteogenic lineage cells could have been induced by the osteogenic induction medium. The up-regulation of alkaline phosphatase and down-regulation of osteocalcin and osteopontin mRNA expression in induced cells compared with un-induced cells indicated that the osteogenic induction medium seemed to play a role in the regulation of the transcription of these specific mRNAs. The high expression of alkaline phosphatase and low expression of osteocalcin and osteopontin also showed that the induced cells might be differentiating into late osteoblasts or pre-osteocytes at the time when total RNA was extracted from the osteogenic culture[14]. Together with the Alizarin Red S staining, we concluded that the MSC were successfully induced to differentiate into the terminal cells of the osteogenic lineage.

In arthritis, the articular cartilage usually has limited capacity to regenerate new cells due to presence of low chondrocytes cellularity and void of blood supply[18]. Wakitani made a defect into the medial femoral condyle at the knee of rabbits and later transplanted a MSC construct in collagen gel. The results showed that the MSC could not only form cartilage, but regenerate into bone at the site of defects.[19] This suggested the potential of MSC to be used for treatment of pathology involving articular cartilage and bone. Osteoarthrosis occurs in 10 to 20 percent of Malaysian population and these patients experienced pain and swelling in the joints causing difficulties in movement. Analgesics and non-steroidal anti-inflammatory drugs have been used for therapy and the last option of treatment would be surgery and replacement with artificial joints.[20] Now, either MSC can be injected directly or the differentiated chondrocytes transplanted into the site of inflammation in the cartilage. MSC is believed to be able reduce the osteoarthrosis symptoms by suppressing the T-lymphocytes proliferation or regenerating new chondrocytes to replace the damaged ones.

Besides manipulation in regenerative medicine, MSC can be used to study the development of human being and to understand the pathways for differentiation. From two studies, osteogenesis of MSC has been found to involve interleukin-6 and gp30 receptors, increase of stat-3 phosphorylation activity, and expression of sortilin.[21,22] However, it is still unclear how much the resemblance of the pathway it is to osteogenesis in embryonic stem cells. Nevertheless, the chondrogenic differentiation of the MSC was found to be similar to the pathways exhibited by the mesenchymal cells during embryonic development[12,23] and thus, MSC can be used as a model to replace embryonic stem cells in the studies of human being development.

With increasing knowledge of MSC properties, we can use the differentiated products as models for studies on sensitivity or reactions towards particular chemical agents, cytokine or growth factor in research and development of pharmaceutical drugs or cell toxicology.[24] There is also suggestion that MSC from individuals can be used to prognosticate disease outcome

or predict treatment response. For example, parathyroid hormone is currently used to increase the osteoblastic activity in the bone. However, the right dose for regiment is difficult to be determined as the anabolic rate of the bone cells is different among individuals. In such case, osteoblast differentiated from the MSC (from the same patient) can be used to test on the sensitivity of the cells to a range of hormone doses, and thus an optimal dose to treat the bone disease can be determined. Besides that, the osteogenic potential of the MSC from an individual can be performed to predict his or her susceptibility to bone disease like osteoporosis or osteoarthritis in the future.[25]

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REFERENCES

1. Rosenthal N. Prometheus's vulture and the stem-cell promise. NEJM 2003; 349(3): 267-74.
2. Friedenstein AJ, Deriglasova UF, Kulagina NN *et al*. Precursors for fibroblasts in different populations of hematopoietic cells as detected by *in vitro* colony assay method. Exp Hematol 1974; 2: 83-92.
3. Hung SC, Chen NJ, Hsieh SL, Li H, Ma HL, Lo WH. Isolation and characterization of size-sieved stem cells from human bone marrow. Stem Cells (Dayt) 2002; 20: 249-58.
4. Qian L, Saltzman WM. Improving the expansion and neuronal differentiation of mesenchymal stem cells through culture surface modification. Biomaterials 2004; 25: 1331-7.
5. Schwartz RE, Reyes M, Koodie L, *et al*. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 2002; 109(10): 1291-302.
6. Petersen BE, Bowen WC, Patrene KD, *et al*. Bone marrow as a potential source of hepatic oval cells. Science 1999; 284: 1168-70.
7. Makino S, Fukuda K, Miyoshi S, *et al*. Cardiomyocytes can be generated from marrow stromal cells *in vitro*. J Clin Inves. 1999; 103(5): 697-705.
8. Jiang Y, Balkrishna NJ, Reinhardt RL, *et al*. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41-9.
9. Mok PL, Leong CF, Cheong SK. Isolation and identification of putative mesenchymal stem cells from bone marrow. Malays J Pathol 2003; 25(2): 121-7.
10. Fielding BA, Frayn KN. Lipoprotein lipase and the disposition of dietary fatty acids. Br J Nutr 1998; 80: 495-502.
11. Mead JR, Irvine SA, Ramji DP. Liporotein lipase: Structure, function, regulation, and role in disease. J Mol Med 2002; 80: 753-69.
12. Imabayashi H, Mori T, Gojo S, *et al*. Redifferentiation of dedifferentiated chondrocytes and chondrogenesis of human bone marrow stromal cells via chondrosphere formation with expression profiling by large-scale cDNA analysis. Exp Cell Res 2003; 288: 35-50.
13. Gartner LP, Hiatt JL. Color atlas of histology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 72-8.
14. Mark KVD. Structure, biosynthesis, and gene regulation of collagens in cartilage and bone. In: Seibel MJ, Robins SP & Bilezikan JP, editors. Dynamics of bone and cartilage metabolism. USA: Academic Press; 1999. p. 3-29.
15. Junqueira LC, Carneiro J. Basic histology: Text & Atlas. 10th ed. USA: Lange Medical Books McGraw-Hill; 2003. p. 135-40.
16. Caterson EJ, Nesti LN, Danielson KG, Tuan RS. Human marrow-derived mesenchymal progenitor cells. Mol Biotechnol 2002; 20: 245-56.
17. Abdul Hamid AR. Anatomi Umum. 2nd ed. Kuala Lumpur: Dewan Bahasa dan Pustaka; 1995. p. 22-46.
18. Tsuchiya H, Kitoh H, Sugiura F, Ishiguro N. Chondrogenesis enhanced by overexpression of sox9 gene in mouse bone marrow derived mesenchymal stem cells. Biochem Biophys Res Commun 2003; 301: 338-43.
19. Wakitani S, Goto T, Pineda SJ, *et al*. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg 1994; 76A(4): 579-92.
20. Yoga SS. Painful joints. The Star 2004 October 27: 10
21. Erices A, Conget P, Rojas C, Minguell J. gp130 activation by soluble interleukin-6 receptor / interleukin-6 enhances osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells. Exp Cell Res 2002; 280(1): 24-32.
22. Maeda S, Nobukuni T, Shimo-Onoda K, *et al*. Sortilin is upregulated during osteoblastic differentiation of mesenchymal stem cells and promotes extracellular matrix mineralization. J Cell Physiol 2002; 193(1): 73-9.
23. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 1998; 238: 265-72.
24. Ong TJ. Use of stem cells in biotherapeutics development. Paper presented at: Adult Stem Cell Workshop. 2003 October 20-23; Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
25. Caplan AI, Bruder SP. Mesenchymal stem cells: Building blocks for molecular medicine in the 21st century. Trends Mol Med 2001; 7(6): 259-63.