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ISOLATION, CHARACTERIZATION, AND DIFFERENTIATION OF MESENCHYMAL STEM CELL FROM RAT BONE MARROW *IN VITRO*

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ABSTRACT

Bone marrow mesenchymal cells have been identified as a source of pluripotent stem cells with varying degrees of plasticity in humans. MSCs have the potential to differentiate into different cell types such as osteoblast, chondroblast, adipoblast. This research describes the pioneering experiment of isolation and differentiation rat bone marrow stromal cells into the osteoblast and adipoblast cells lineages. Bone marrow stromal cells were isolated from long bones of rat, based on the adherent properties of the MSC. The stromal cells obtained by direct plastic adherence were characterized by fluorescent activating cell sorting (FACS) for established hematopoietic and non-hematopoietic markers. Under the determinate effect of culture conditions, MSCs were differentiate done osteogenic and adipogenic cell lines detected by Alizarin red and oil-red O staining invitro. Bone marrow samples from rats yielded 4-5 million bone marrow mononuclear cells/ml per femur. The MSCs culture is being investigated with microscopic observations. They form elongated or spindle-shaped fibroblast-like cells. Cell sorting by the surface marker expression is used in the current studies for confirmation of rat MSCs in the culture. Moreover, the cells differentiated toward osteoblasts and adipocytes were characterized morphologically by Alizarin Red staining and Oil Red O staining. This research consigns new ideas to develop noble markers and play a vital role in tissue engineering.

KEY WORDS

MSCs, FACSMSCs, Osteogenic, Adipogenic differentiation.

INTRODUCTION

Bone marrow is the main source of stem cells (SCs) especially mesenchymal stem cells (MSCs). HSCs can be found in adult bone marrow which is regulated within the microenvironment of the stromal cells of the bone marrow [1]. MSCs are multipotent cells that can differentiate into various cell lineage depending upon their environment and culture condition in which they are preserved [2]. MSCs differentiation potential is retained even after repeated sub culturing *in-vitro* condition [3-4] MSCs could differentiate into various non-mesenchymal tissue lineages under appropriate experimental conditions *in-vitro* and *in-vivo*, such as hepatocytes [5-6] cardiomyocytes[7-8] lung alveolar epithelium[9] olfactory epithelium[10], inner hair cells[11], neurons and neuroglia[12-14].

MSCs have also been named colony-forming fibroblastic cells [15], marrow stromal stem cells [16-17] and mesenchymal progenitor cells [18]. These cells are considered to be an appropriate source of cell and gene therapy tools for treatment in a number of congenital degenerative diseases, long-term self-renewing,

capabilities of pluripotency including osteoblast, adipocytes, chondrocytes, tenocytes, muscle cells and neurogenic cells, which make them ideal source of stem cells for regeneration of injured tissue [19-21].

MSCs was first described as bone forming progenitors from the stromal fractions of rats [22] and Friedenstein *et al.* 1987[23] went on to pioneer *invitro* culture method for isolation and differentiation of MSCs. Bone marrow-derived MSCs have been isolated from a variety of species, including human [24] mouse [25] rat [26-27] dog, baboon, pig, sheep, goat, rabbit [28] and cat[29]. While MSCs from different organisms have similar characteristics in part, some data suggest that variations occur among species. The most collected MSCs are spindle-shaped fibroblast-like cells that are easily isolated, cultured and expanded *in vitro* due to their adherent characteristics, and not associated with any ethical debate [30].

The present study describes an evident method of establishing the rat bone marrow stromal cells by the principle of adhesion, and differentiation potential to other lineages adipo-inductivity, osteo-inductivity and possible for application in animal biotechnology.

MATERIALS AND METHODS

Animals

Adult male Wistar (albino) rats weighed between 150 g were obtained from the animal house of Kongunadu Arts and Science, Coimbatore, India. The animal study was followed by according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal International Ethical Committee approved added experimental design performed in this study for the use of Wistar albino rats as an animal model for isolation of MSCs. The animals were fed with standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water freely available throughout the experimental period and refilled daily. The animals were housed in well ventilated large polypropylene cages under controlled conditions of light (12 hr light/12 hr dark), humidity (50-55 %) and ambient temperature (25 ± 2°C).

MSC Isolation and Culture

The mouse was sacrificed by decapacitation (cervical dislocation). Remove the muscles tissue from the femur and tibia, separate femur and tibia and cut off the epiphysis on both ends. With the help of syringe (21G needle), the bone marrow was aseptically extruded with 5 ml PBS solution and flushed out (10 times). The marrow tissue was dissociated by pipetting. Wash the suspension by centrifugation at 500 RPM for 5 minutes. Discard the supernatant. MSCs were mechanically dispersed into a single-cell suspension and the yield of cells is 10^6 cells/ml. At this point, marrow cells were plated in a 25 cm² plastic flask in DMEM containing 20% FBS, 100 U/ml penicillin, and 100 mg/ ml streptomycin. All the cells were incubated at 37°C, in an atmosphere of 5 % humidified CO_2. After 48 hours of incubation, the non-adherent cells were removed. The medium was added and replaced every 3 to 4 days for about two weeks. When the cells reached 80% confluency, they were harvested in 0.25% trypsin and 1 mM EDTA for 5 min at 37°C. The cells were replaced and diluted in the ratio of1:3 on a 25 cm² plastic flask. This procedure was repeated for the next confluence. MSCs were passaged approximately 15 times and morphologically evaluated prior to use in inducing differentiation and vitrification.

Test of cell viability

Before culturing, the trypan blue dye exclusion test is used to determine the number of viable cells present in a cell suspension. The dye was diluted with PBS (0.4 trypan blue /PBS) and equal parts of trypan blue dye were added to the 100ul cell suspension. As a result, the dye was not absorbed by viable cells.

The culture of separated mononuclear cells from rat

The mononuclear cell suspension obtained was resuspended in complete high glucose Is cove's Modified Dulbecco's Medium containing 4.5 g/L glucose with L-glutamine,10% FBS, 1% penicillin and streptomycin - Amphotericin mixture. Cells were cultured at a concentration of $5*10^6/25cm^2$ culture flask. These were incubated in a 5% humidified CO_2 incubator at 37°C. To remove non-adherent cells the medium was replaced every 3-4 days. When large colonies developed (80-90% confluence), cultures were washed twice with PBS and cells were trypsinized using 0.25% trypsin/ EDTA for 5 min at 37ºC. After centrifugation at 2400 RPM for 20minutes. The cell cultures recovered after 7days are referred to as "first passage cultures".

FACS Analysis

Flow cytometric was performed in FACS caliber. The cells were centrifuged at 1200 RPM for 5 min after trypsinization the pellet was dissolved with 1x 10^6 /ml PBS concentration. The cells were incubated with phycoerythrin - or fluorescein isothiocyanate-conjugated antibodies against CD90, CD73, CD105,

CD45, CD14, and CD34 surface and negative markers. All analyzed standard cells were incubated with isotype-specific IgGs at room temperature for 40 min. The cells were washed twice with PBS containing 2% bovine serum albumin and centrifuged at 1500 RPM for 5 min. To each tube 50ul of cell suspension was added ($1x10^6$/ml) and incubated for 15min at room temperature in the dark environment. After 15min, PBS was added in all the tubes and made up to 1ml and centrifuged at 1200 RPM for 5 min. The supernatant was discarded. 250ul PBS was added in all the tubes. Now the tubes are subjected to FACS analysis. Tubes were acquired serially. Before recovering, propidium iodide (2ul) was added to analyze the viability of the cells in the sample.

Adipogenic induction

MSCs were placed on a coverslip and cultured in a six-well plate containing DMEM with 10% FBS. Cells with nearly 80% confluency were exposed to DMEM supplemented with 5µg/ml insulin, 1µM dexamethasone, 100nM indomethacin, 0.5 mM methyl isobutyl xanthine, and 10% FBS for 48 hours. These were incubated in the same medium without dexamethasone. For control, cells were cultured in the regular medium as mentioned above. The medium was replaced every third or fourth day. One week after the induction, adipogenic differentiation was evaluated by the cellular accumulation of neutral lipid vacuoles that were stained with oil-red O and observed under an inverted microscope. After fixation in 5% methanol, induced MSCs were stained in filtered oil red O for 2-3 hours and rinsed with 60% isopropyl alcohol.

Osteogenic induction

MSCs were placed on a coverslip and cultured in a six-well plate containing DMEM-Low Glucose supplemented with 10% FBS, 100nM dexamethasone, 10mM ß-glycerol phosphate, and 50µM ascorbic acid-2-phosphate in 400µl for subsequent staining. During the culture period, the medium was changed once per week. After 14 days, osteogenic differentiation was evaluated by staining the coverslips with fresh 0.5% alizarin red solution.

RESULT

Isolation and culturing of MSCs

By plastic adherence, the cells suspension containing both stromal and hematopoietic cells was seeded in tissue culture flasks using DMEM with 10% FBS. At the end of the seventh day, rounded and spindle-shaped cells had attached to the bottom of the tissue culture flask (Figure 1A). Even on subsequent media change most of these rounded cells remained adherent. The spindle-shaped fibroblast-like cells (Figure 1B) from rat bone marrow were purified by a repeated medium change at initial hours of culture and diminishing the trypsinization time. The cells were observed under confocal microscopy. The cell viability was checked with trypan blue and viable cells were counted. Trypan blue stains the dead cells and the viable cells were observed as colorless. The result shows that viable cells are dominant than the dead cells. The proliferation of MSCs was in passage 2 or 3 during culture initiation, the MSCs harvest was adapted after 10 passages. MSC cells were expanded into round cells and eventually turns spindle-shaped forming colonies of fibroblast when plated in the low density.

Characterization of cultured MSCs by flow-cytometry

Flow cytometric analysis of cell surface markers in mesenchymal stem cells expressed CD90, CD73, CD105, CD45, CD14, and CD34. The surface markers expression pattern corresponds to the bone marrow-derived mesenchymal stem cells.

Differentiation

Both types of MSCs successfully differentiated into adipocytes and osteoblasts lineages (Figure 2 A, B, and C). The negative control (non-induced) cells for each type of differentiation showed negative for Alizarin Red and Oil Red O stains. Osteogenesis of Bone marrow-derived MSCs demonstrates mineralized matrix 21 days after induction. Adipogenesis of Bone marrow-derived MSCs was detected by the formation of lipid droplets stained with Oil Red O staining, 21 days after induction.

FACS Results

Mesenchymal stromal cells are a good resource for regenerative medicine because of their diverse cellular differentiation potential and trophic effects [31]. Bone marrow has been studied extensively as a source of MSCs.

Although there is an increasing number of reports describing the presence of MSCs. only a few markers have been established to identify these cells in the respective organs. In previous reports, MSCs have been found to be difficult to isolate and are usually contaminated by hematopoietic precursors. Typical hematopoietic surface markers such as CD 14, CD 34, and CD45 (figure 3, 4 and 5) were used as negative markers [32]. Our result also confirms that MSC s will be

negative for all the hematopoietic markers namely (Figure 7) CD 14, CD 34 and CD45 which expressed in M1 (Figure 6), not in M2. There are some positive markers for MSC s which will confirm the presence of MSCs. The positive markers like CD 73 (Lymphoid tissue) CD 90 (T cells, granulocytes, early hematopoietic cells, epithelium, fibroblasts, neurons) CD105 (Endothelium, syncytiotrophoblast, macrophages, fibroblasts) cell surface antigens were analyzed by the group of researchers [33-36].

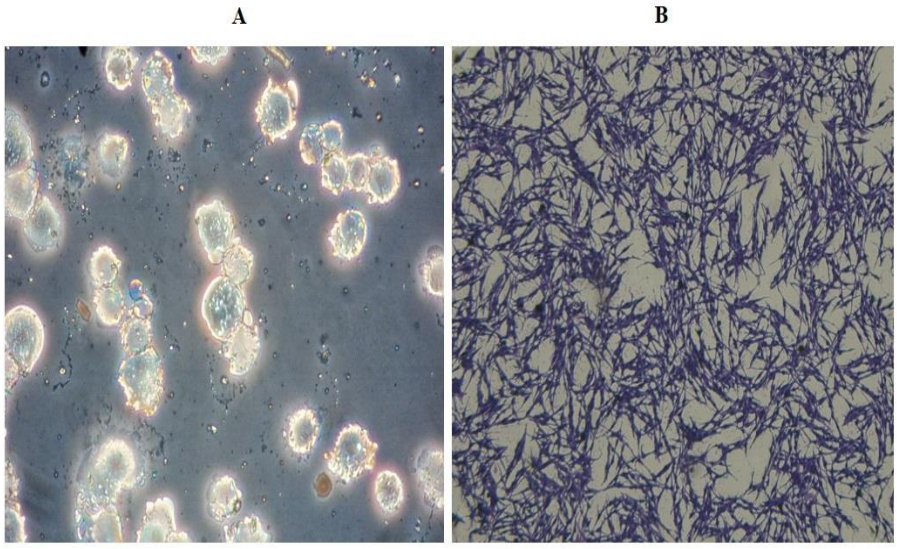


Figure 1 (A)Cultured rat MSCs after 7 days, (B) Cultured MSCs after 21 days

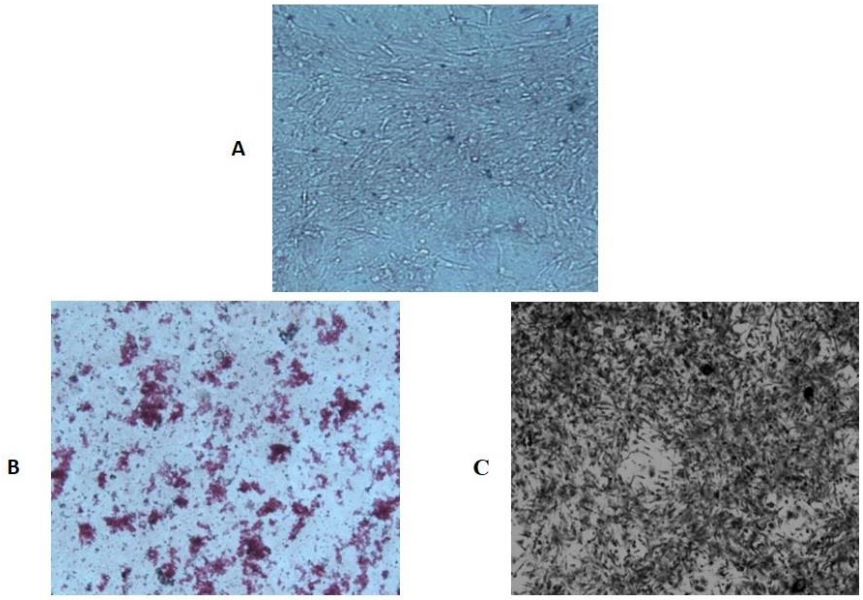


Figure 2 (A). Cultured MSCs exhibited flat morphology, (B). Appearance of adipocytes displayed dark red specific staining for Oil O Red at passage 11, (C). Appearance of Osteocytes displayed a grey colour for specific stain Alizarin red at passage 11.

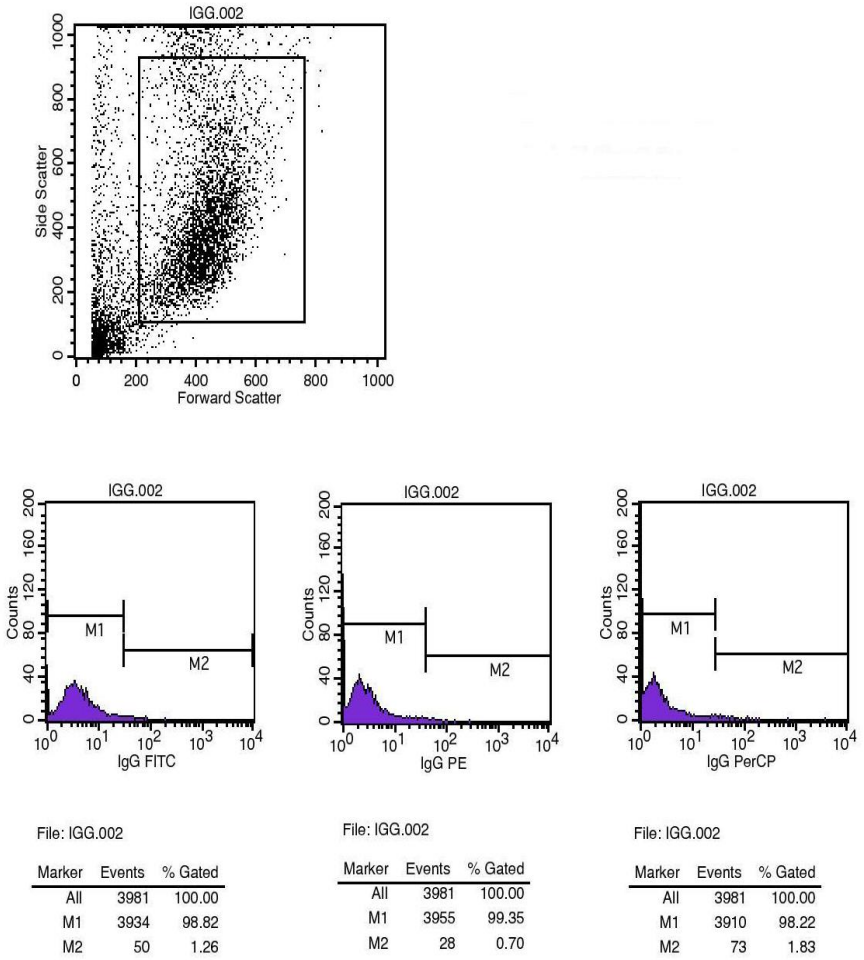


Figure 3 Flow cytometry graph expressing forward scatter of harvested rat MSCs and also IgG FITC, IgG PE, IgG PerCP

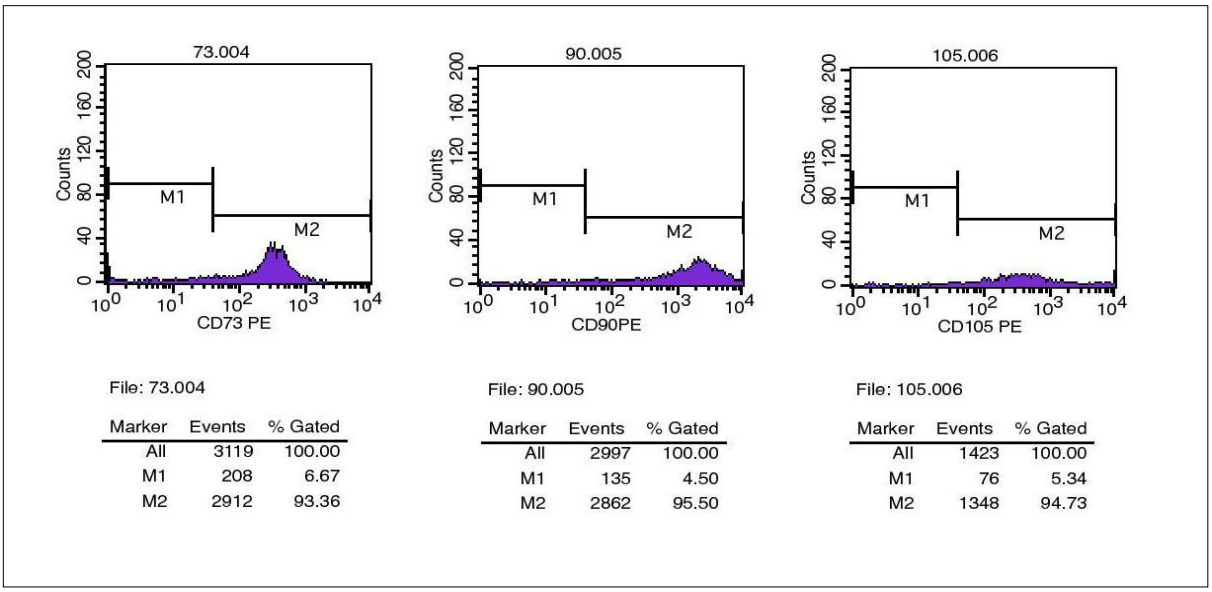
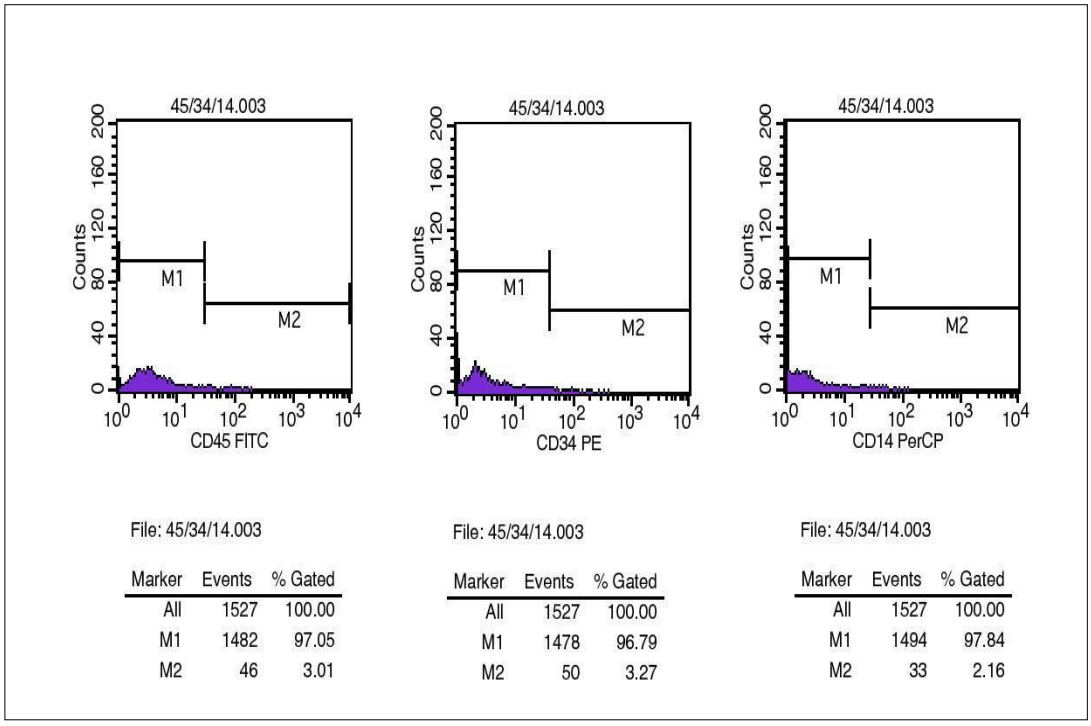


Figure 4 The immunotyping results showed a positive for MSC surface markers namely CD73PE, CD90PE, and CD105PE. The result was found in M2 -10^3- 10^5, which was not expressed in M1 (10^0- 10^{-2})



File: 45/34/14.003

Marker	Events	% Gated
All	1527	100.00
M1	1482	97.05
M2	46	3.01

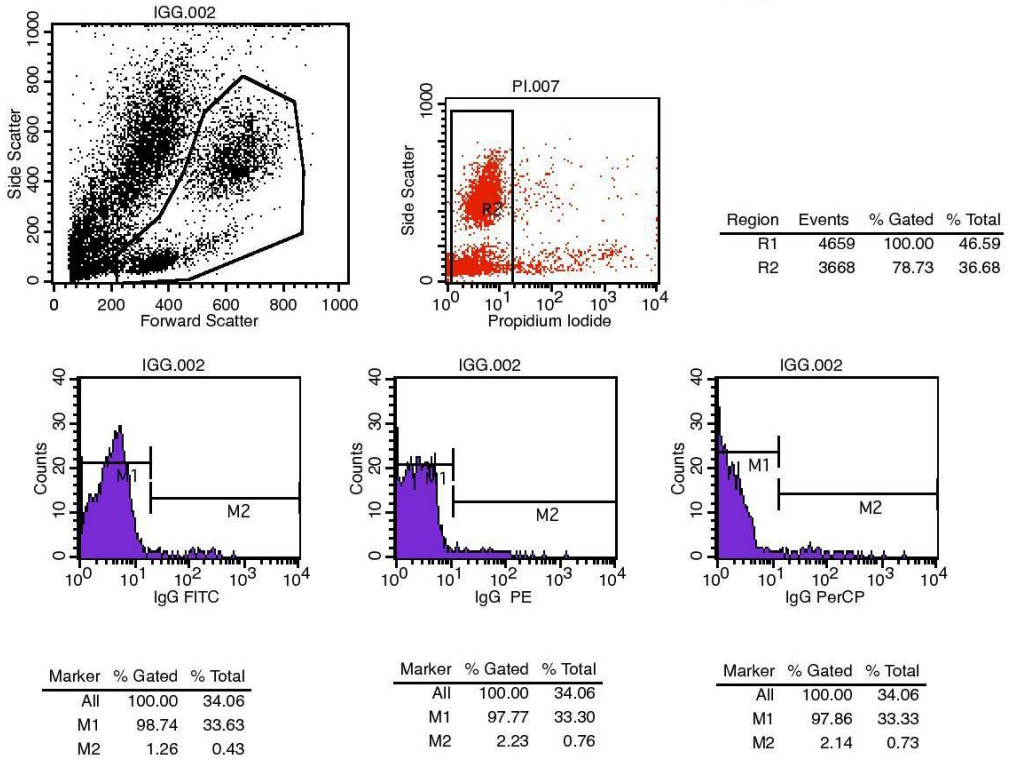
File: 45/34/14.003

Marker	Events	% Gated
All	1527	100.00
M1	1478	96.79
M2	50	3.27

File: 45/34/14.003

Marker	Events	% Gated
All	1527	100.00
M1	1494	97.84
M2	33	2.16

Figure 5 The immunotyping of CD45FITC, CD34PE, and CD14 PerCP - Hematopoitic markers revealed negative result, which was in 10^0-10^{-2} (M1)



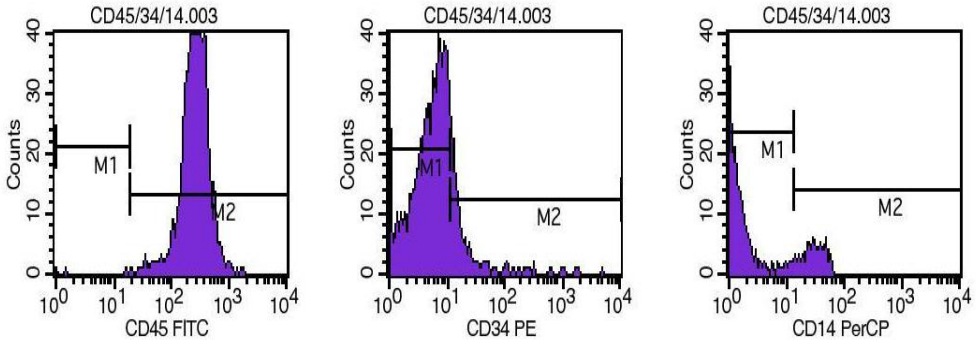
Region	Events	% Gated	% Total
R1	4659	100.00	46.59
R2	3668	78.73	36.68

Marker	% Gated	% Total
All	100.00	34.06
M1	98.74	33.63
M2	1.26	0.43

Marker	% Gated	% Total
All	100.00	34.06
M1	97.77	33.30
M2	2.23	0.76

Marker	% Gated	% Total
All	100.00	34.06
M1	97.86	33.33
M2	2.14	0.73

Figure 6 Initiation of MSCs exhibiting forward scatter, propodium iodide stain IgG FITC, IgG PE, IgG PerCP exhibited results in M1

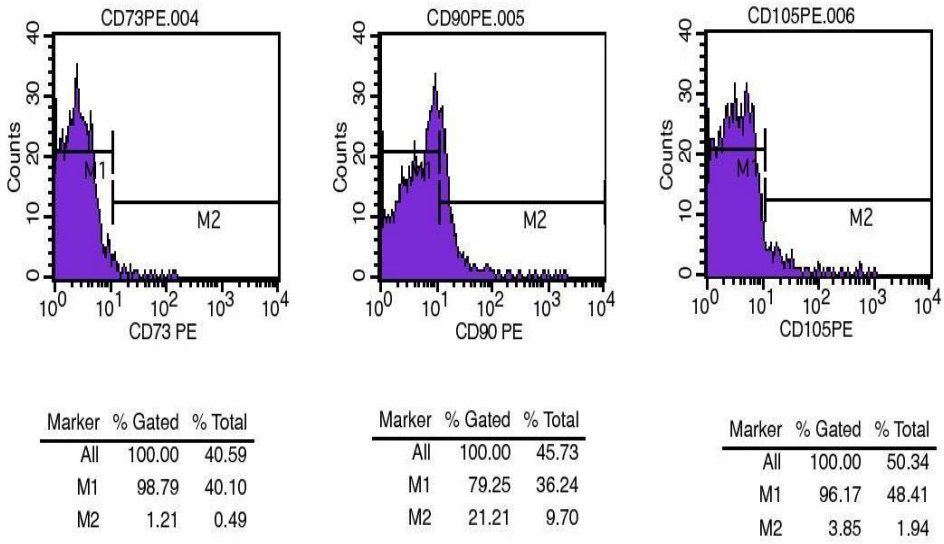


Marker	% Gated	% Total
All	100.00	48.44
M1	0.10	0.05
M2	99.90	48.39

Marker	% Gated	% Total
All	100.00	48.44
M1	86.52	41.91
M2	13.87	6.72

Marker	% Gated	% Total
All	100.00	48.44
M1	94.43	45.74
M2	5.57	2.70

Figure 7. Initiation of MSCs exhibiting CD45 FITC, CD34 PE, CD14 PerCP markers



Marker	% Gated	% Total
All	100.00	40.59
M1	98.79	40.10
M2	1.21	0.49

Marker	% Gated	% Total
All	100.00	45.73
M1	79.25	36.24
M2	21.21	9.70

Marker	% Gated	% Total
All	100.00	50.34
M1	96.17	48.41
M2	3.85	1.94

Figure 8 Initiation of MSCs exhibiting CD 73, CD90, and CD 105 Markers.

DISCUSSION

Bone marrow has an extremely complex cellular arrangement of bone marrow stroma, to maintain the hemopoietic microenvironment. Mesenchymal stromal cells are a good resource for regenerative medicine because of their diverse cellular differentiation potential and trophic effects [37-38]. Bone marrow-derived MSCs possess considerable potential towards the development of cell-based therapeutics. MSCs can differentiate not only into mesenchymal lineage cells but also into various other cell lineages. As MSCs can easily be isolated from bone marrow, they can be used in various tissue engineering strategies [39]. The present study deals with isolation and differentiation of bone marrow-derived MSCs from a rat.

Inconsistent results were obtained by various authors during Isolation of MSC, as because isolation of MSC varied dramatically due to their species difference and adopting methodologies for expansion and plating of cells [40-41]. Due to plastic adherence nature of stem cells can be obtained easily. However, recovery of pure stromal cells is difficult. The present study (figure 1 A, B)

showed MSCs were successfully isolated from the bone marrow and were cultured for 20 passages displaying stable and consistent growth rates. Spindle-shaped fibroblast-like cells from rat bone were observed under confocal microscopy. The cell viability was checked with trypan blue and viable cells were counted. The result shows that viable cells are dominant than the dead cells. MSC cells had spindle-shaped morphology and forming colonies of fibroblast when plated in the low density. The proportion of flattened cells in relation to the other distinct cell morphologies observed increased gradually with time, and usually, some degree of morphological heterogeneity could still be observed up to passages 8-9[42]. The present result investigates and confirms the presence of MSC s, it's full fill the criteria for the presence MSC precursors, the results were positive for CD 73, CD 90, CD 105, which was shown in Figure 8. The recent study in the human MSCs reports the similar observations [43].

Cultured MSCs are known to typically express the cell surface markers CD13, CD73, CD90, CD105, andCD166, and lack the expression of CD34, CD45, CD133, and HLA-DR [44]. The cultured cells were investigated for expression of several markers' characteristic for MSC at passages 2 and 10. We checked the transcription of CD14, CD34 and CD45, which have been indicated to be absent on MSC as well, CD105 which are typical markers expressed by MSC [45-46].

Recent reports show that MSCs might have the ability to differentiate into other lineage cells in vitro. In vivo studies have also shown that MSCs can differentiate into tissue-specific cells in response to cues provided by different organs [47]. Cell sorting by surface marker expression was used in the present study for confirmation of rat MSCs in the culture. Immunophenotyping the surface marker profile of murine MSCs is compatible with that of murine bone marrow stromal cells [48], murine stromal cell lineages [49] and human MSCs [50].

The isolated stromal cells showed differentiation into o ther mesenchymal lineages adipogenic and osteogenic as observed earlier groups [51-53]. In our study, we indicate that rat MSCs retain the capacity to differentiate into mesenchymal derivatives, specifically osteogenic and adipogenic cells.

MSCs may be useful in treating a wide variety of diseases, offering significant advantages over other Stem cells. Final results of our study reveal that we have developed a new protocol for isolation and differentiation of MSCs from the heterogeneous mixture of bone marrow cells, mainly based on a frequent medium change in primary culture and diminishing the trypsinization time.

CONCLUSION

In conclusion, our study indicates that using a simple principle of adhesion it is possible to isolate rat MSCs and growth. Exposing to the osteogenic and adipogenic factors we were able to differentiate and characterize them into the osteoblast and adipoblast like cells in vitro. This experimental protocol of the rat MSC culture system can be used for investigation of the wide variety of metabolic or re-generative bone diseases.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS USED

FACS: fluorescent activating cell sorting; **MSCs**: Mesenchymal stem cells; **DMEM**: Dulbecco's modified eagle medium; **EDTA**: Ethylenediaminetetraacetic acid; **PBS**: Phosphate buffered saline; **FBS**: Fetal bovine serum: **RPM**: Rotation per minute.

REFERENCE

1. Pontikoglou C, Deschaseaux F, Sensebé L, Papadaki HA. Bone marrow mesenchymal stem tcells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. Stem Cell Reviews and Reports, 7(3):569-89, (2011).
2. Tae SK, Lee SH, Park JS, IM GI. Mesenchymal stem cells for tissue engineering and regenerative medicine. Biomed Mater, 1(2):63-71, (2006).
3. Lee MW, Choi J, Yang MS, Moon YJ, Park JS, Kim HC, Kim YJ. Mesenchymal stem cells from cryopreserved human umbilical cord blood. BiochemBiophys Res Commun, 320(1):273-8, (2004a).
4. Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified

human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem, 64(2):278-94, (1997).
5. Kang XQ, Zang WJ, Song TS, Xu XL, Yu XJ, Li DL, Meng KW, Wu SL, Zhao ZY. Rat Bone marrow mesenchymal stem cells differentiation into hepatocytes in vitro. World J Gastroenterol, 11(22):3479-84, (2005).
6. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature, 418:41-49, (2002).
7. Baharv and H, Mathae KI. Culture condition difference for the establishment of new embryonic stem cell lines from the C57 BL/6 and BALB/c mouse strains. In Vitro Cell Dev Biol Anim, 40(3-4):76-81, (2004).
8. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation, 105:93-98, (2002).
9. Kotton DN, Ma BY, Cardoso WV, Sanderson EA, Summer RS, Williams MC, Fine A. Bone marrow-derived cells as progenitors of lung alveolar epithelium. Development, 128:5181-88, (2001).
10. Tsujigiwa H, Nishizaki K, Teshima T, Takeda Y, Yoshinobu J, Takeuchi A, Orita Y, Sugata Y, Nagatsuka H, Nagai N. The engraftment of transplanted bone marrow-derived cells into the olfactory epithelium. Brain Res, 1052(1):10-15, (2005).
11. Jeon SJ, Oshima K, Heller S, Edge AS. Bone marrow mesenchymal stem cells are progenitors in vitro for inner ear hair cells. Mol Cell Neurosci, 34(1):59-68, (2007).
12. Eslaminejad MB, Nazarian H, Taghiyar L. Mesenchymal stem cells isolation from the removed medium of rat's bone marrow primary culture and their differentiation into skeletal cell lineage. Yakhteh, 10(1):62-72, (2008).
13. Lee MW, Choi J, Yang MS, Moon YJ, Park JS, Kim HC, Kim YJ. Mesenchymal stem cells from cryopreserved human umbilical cord blood, BiochemBiophys Res Commun, 320(1):273-8, (2004b).
14. Suzuki H, Taguchi T, Tanaka H, Kataoka H, Li Z, Muramatsu K, Gondo T, Kawai S. Neurospheres induced from bone marrow stromal cells are multipotent for differentiation into the neuron, astrocyte, and oligodendrocyte phenotypes. Biochem Biophys Res Commun, 322(3):918-22, (2004).
15. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol, 4:267-74, (1976).
16. Bianco P, Riminucci M, Gronthos S, and Robey PG. Bone marrow stromal stem cells: Nature, Biology, and potential applications. Stem cells, 19:180-92, (2001).
17. Prockop DJ. Marrow Stromal cells as stem cells for non-hematopoietic tissues. Science, 276:71-74, (1997a).
18. Sun S, Guo Z, Xiao X, Liu B, Liu X, Tang PH, Mao N. Isolation of mouse marrow mesenchymal progenitors by a novel and reliable methods. Stem cells, 21:527-35, (2003).
19. Baksh D, Song L, and Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med, 8:301-16, (2004).
20. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol, 213(2):341-347, (2007).
21. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. Stem Cells Dev, 21(14):2724-52, (2012).
22. Friedenstein AJ, Piatetzky S, Potrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryop Exp Morphol, 16:381-90, (1966).
23. Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: in-vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet, 20:263-72, (1987).
24. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science, 284:143-7, (1999a).
25. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Chaser (CSR), a new gene affecting larval foraging behavior in Drosophila melanogaster. Genetics, 141(1):263-70, (1995).
26. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle and Nerve, 18:1417-26, (1995).
27. Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplantation, 6:125-34, (1997).
28. Mosca JD, Hendricks JK, Buyaner D, Davis-Sproul J, Chuang LC, Majumdar MK, Chopra R, Barry F, Murphy M, Thiede MA, Junker U. Mesenchymal stem cells as vehicles for gene delivery. Clinical Orthopaedics and Related Research, 379S:S71-90, (2000).
29. Martin DR, Cox NR, Hathcock TL, Niemeyer GP, Baker HJ. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. Experimental Hematology, 30:879-86, (2002).
30. Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M, Lanzoni G, Cantoni S, Cavallini C, Bianchi F, Tazzari PL. Term Amniotic membrane is high throughout source for multipotent mesenchymal stem cells with the

ability to differentiate into endothelial cells in vitro. BMC Dev Biol, 7:11, (2007).
31. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem, 98:1076-84, (2006a).
32. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science, 284:143-7, (1999b).
33. Barry FP, Boynton RE, Haynesworth S, Murphy JM, Zaia J. The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). Biochem Biophys Res Commun, 265:134-139, (1999).
34. Barry F, Boynton RE, Liu B, Murphy JM. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. Exp Cell Res, 268:189-200, (2001).
35. 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, 20:249-258, (2002).
36. De Ugarte DA, Alfonso Z, Zuk PA, Elbarbary A, Zhu M, Ashjian P, Fraser JK. Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. Immunol Lett, 89:267-270, (2003).
37. Polisetti N, Chaitanya VG, Babu PP, Vemuganti GK. Isolation, characterization and differentiation potential of rat bone marrow stromal cells. Neurol India, 58:201-8, (2010).
38. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem, 98:1076-1084, (2006b).
39. Mikako S, Riichiro A, Yasuyuki F, Satomi A, Daisuke I, Hiroshi S. Mesenchymal Stem Cells are Recruited into Wounded Skin and Contribute to Wound Repair by Transdifferentiation into Multiple Skin Cell Type. J Immunol, 180:2581-7, (2008).
40. Bernacki SH, Wall ME, Loboa EG. Isolation of human mesenchymal stem cells from bone and adipose tissue. Methods Cell Biol, 86:257-78, (2008).
41. Cheng MT, Yang HW, Chen TH, Lee OK. Isolation and characterization of multipotent stem cells from human cruciate ligaments. Cell prolif, 42(4):448-60, (2009).
42. Tremain N, Korkko J, Ibberson D, Kopen GC, DiGirolamo C, Phinney DG. MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. Stem Cells, 19(5):408-18, (2001).
43. Chase LG, Lakshmipathy U, Solchaga LA, Rao MS, Vemuri MC. A novel serum-free medium for the expansion of human mesenchymal stem cells. Stem Cell Res Ther, 1:8, (2010).
44. Caplan AI. Mesenchymal stem cells. J Orthop Res, 9:641-650, (1991).
45. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of trans differentiation and modes of tissue repair—current views. Stem cells, 25:2896-2902, (2007).
46. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science, 284:143-7, (1999c).
47. Silva Jr WA, Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JL, Zanette DL, Santos AR, Zago MA. The profile of gene expression of human marrow mesenchymal stem cells. Stem Cells, 21(6):661-9, (2003).
48. Lindolfo da Silva Meirelles and Nance Beyer Nardi. Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization. British Journal of Haematology, 123:702-11, (2003).
49. Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by trans differentiation into multiple skin cell type. J Immunol, 180:2581-7, (2008).
50. Wieczorek G, Steinhoff C, Schulz R, Scheller M, Vingron M, Ropers HH, Nuber UA. Gene expression profile of mouse marrow stromal cells determined by cDNA microarray analysis. Cell and Tissue Research, 311:227-37, (2003).
51. Prockop DJ. Marrow Stromal cells as stem cells for non-hematopoietic tissues. Science, 276:71-74, (1997b).
52. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem, 98:1076-84, (2006c).
53. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science, 284:143-7, (1999d).

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