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Role of Visualization in Stem Cell Characterization

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Abstract : Stem cells are very promising for regenerative medicine due to their self renewal and differentiation potentials. To be used for patients the stem cells should be fully characterized. Characterization of the stem cells includes visualization of cell morphology whether in fresh conditioned, in situ staining or staining of cell suspension. Moreover, visualization gives an insight of cell property whether the cells are adherent or non adherent, and whether the cells are forming clones or a monolayer. Surface marker or senescent staining combined with visualization may determine the type of stem cell or senescent condition, and visualization of induced stem cells can show their differentiation capacity.
Keywords: staining, stem cell, characterization, differentiation, senescence.

Introduction

Stem cells are very promising for regenerative medicine due to their self renewal and differentiation potentials. A growing body of evidence showed that stem cells might cure various kind of degenerative diseases or conditions involving cell/tissue damages such as stroke, myocardial infarct, liver cirrhosis, ankylosing spondylitis, osteoarthritis, diabetes, and inherited disease such as thalasemia.[1-6] Stem cells can be used in various methods, including cell replacement therapy, activation of resident stem cells by paracrine secretion, and tissue engineering.[7] There are various kinds of stem cells, and characters of those different stem cells need to be elucidated to regard them as a certain stem cell, e.g. mesenchymal,[8] hematopoietic,[9] and neural stem cell.[10, 11]

Stem cell based therapy needs a lot of stem cells, and thus the stem cells need to be propagated by culturing them to certain passages. Culture conditions and passage number might influence the character of the stem cells. Therefore, to be used for patients the stem cells should be fully characterized. Characterization of stem cells involved various criteria, including cell morphology, property, surface markers, differentiation capacity, and senescence. For the purpose of characterization, visualization is an important part, especially in stem cell mass production that involved high passage number. Therefore, this mini review deals with the role of visualization in various aspect of stem cell characterization that is indispensable in stem cell production.

Visualization of cell morphology and property

In stem cell characterization, visualization plays a substantial role to elucidate cell morphology, which can be conducted in fresh conditioned (Figure 1A, 1B), in *in situ* staining (Figure 2) or staining of cell suspension. Visualization of cell suspension after harvesting can be done by making spot specimens, which can be stained. Using this method, change in morphology due to differentiation can be visualized (Figure 3).[12] Further, using a special program, the outline of cells can be demarcated so that the area (size) of the cells can be

computed (Figure 4).[13] Under the microscope cell property can be visualized, whether the cells are adherent to the culture plate (Figure 1A, 1B) or non adherent (Figure 5), forming clones (Figure 6), monolayer, multiple layer, micro masses (Figure 7), or forming spheres such as in cancer stem cells.[14] Morphology of adherent cells in culture might be fibroblastic such as fibroblasts and mesenchymal stem cells (MSCs) (Figure 1A); diamond shape that suggests epithelial cells such as keratinocytes (Figure 1B); neuron like, etc. Sprouting of MSCs from explants can also be visualized, [15] and visualization is also useful to monitor viable cells when stem cells are grown on a scaffold.[16, 17]

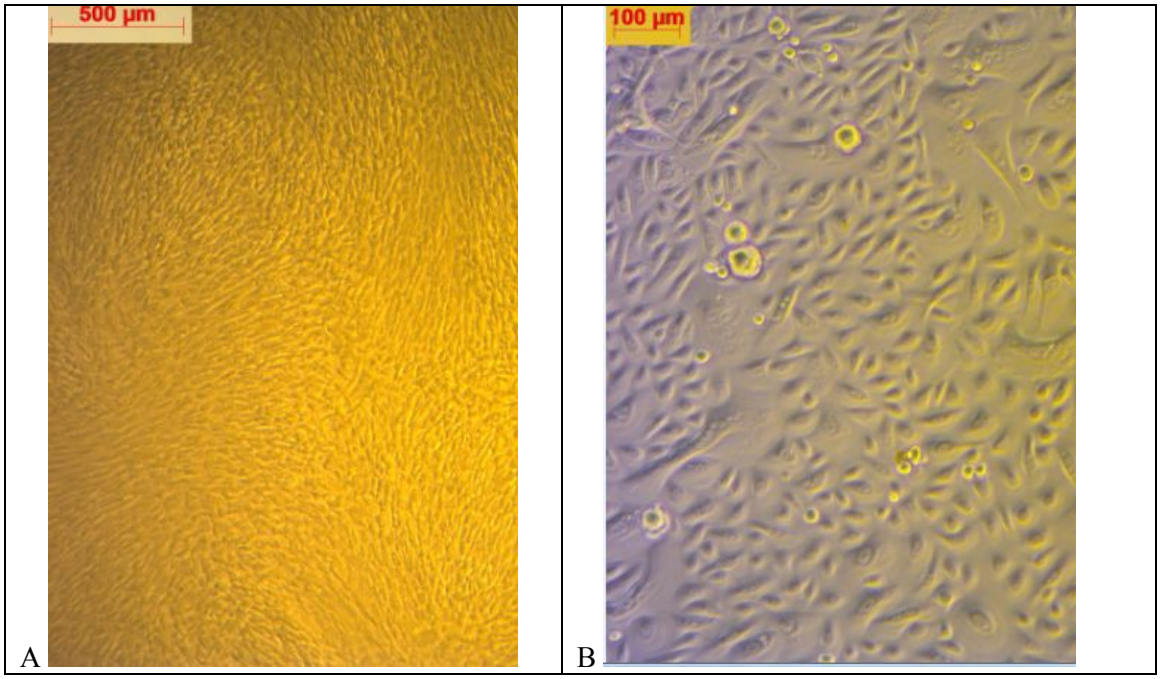

Figure 1. Cell morphology of attached cells in fresh condition (Nikon inverted microscope and Axiocam digital microscope camera)
A. Adipose derived MSCs at 95% confluent, B. Foreskin derived keratinocytes.

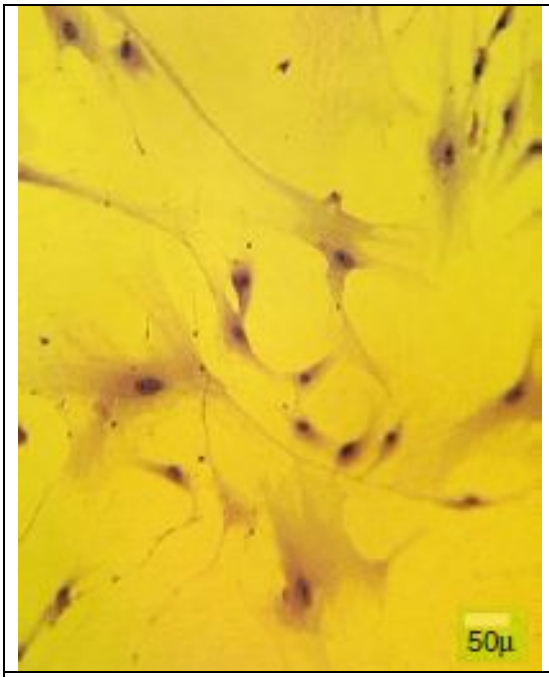

Figure 2. In situ Giemsa staining of adipose derived stem cells (Nikon inverted microscope and Nikon camera) [18]

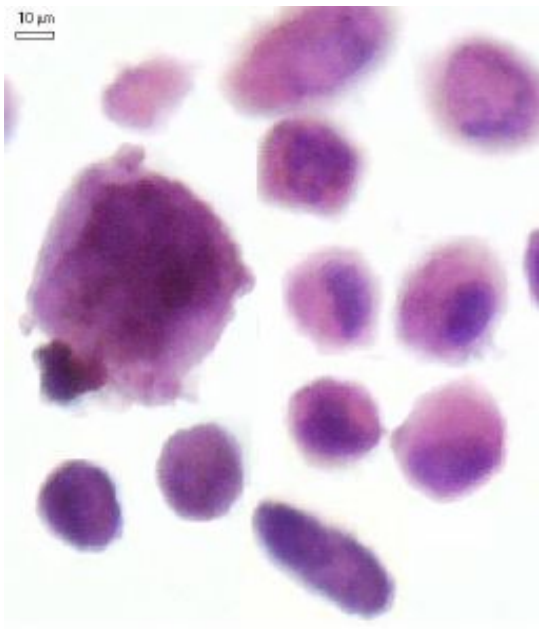

Figure 3. Giemsa staining of adipose derived stem cells in spot method (light microscopy and Optilab digital camera) [19]

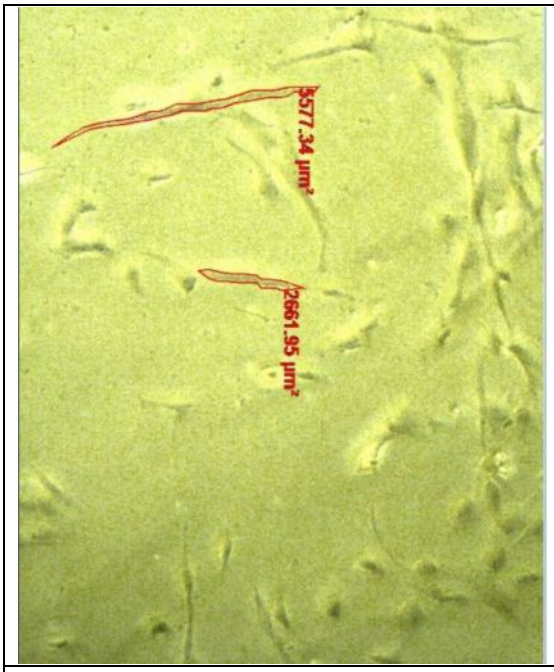

Figure 4. Cell size computation (Axiocam computer program) [13]

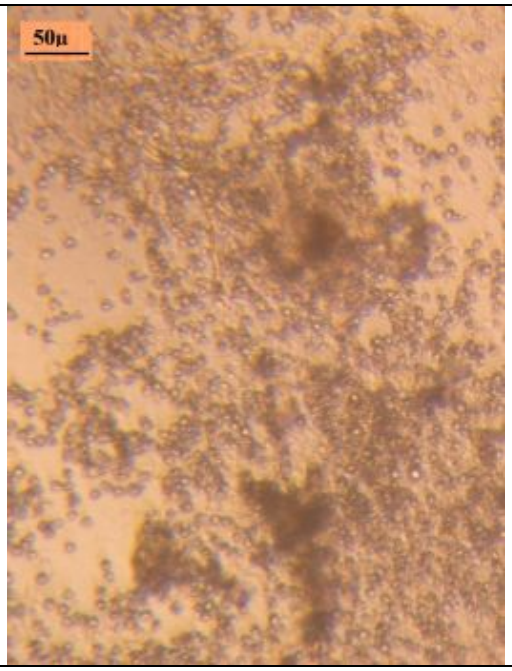

Figure 5. Non adherent stem cells (Nikon inverted microscope and Axiocam digital microscope camera)

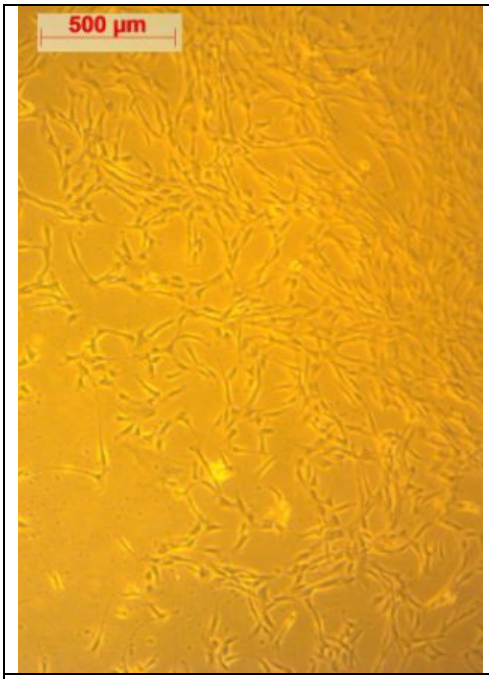

Figure 6. A large clone of bone marrow derived MSCs (Nikon inverted microscope and Axiocam digital microscope camera)

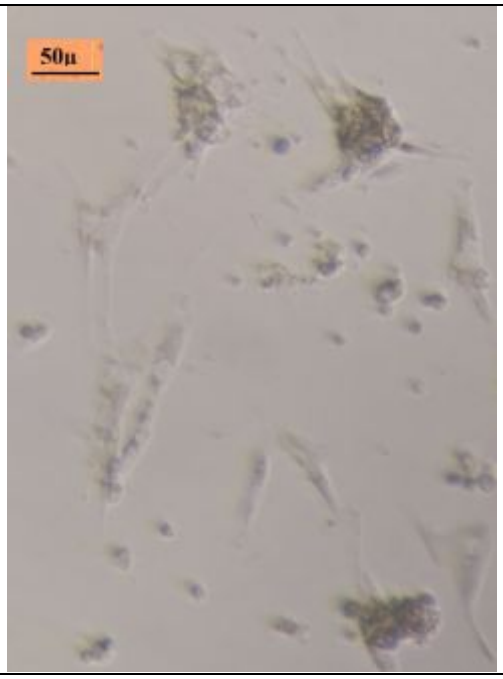

Figure 7. Micro masses of adipose derived stem cells (Nikon inverted microscope and Nikon camera)

Adherent to plastic is a special property of MSCs, when they are cultured in serum containing medium, [20] as serum contains attachment factor. However, when commercial media are used, most commercial media do not contain attachment factors, and for MSCs to grow, the culture flask/plate should be coated by extracellular matrix, such as collagen, fibronectin, or laminin.[21] On the contrary, hematopoietic stem cells are non adherent and appear floating in the medium (Figure 5).[22]

When MSCs or other attached cells are cultured in a few numbers, they will form clones that derived from one single cell. This clone forming property is usually used to assess the number of stem cells in a tissue derived suspension, such as stromal vascular fraction or processed lipoaspirate, namely the colony forming unit assay, or serial dilution assay.[23] However, when MSCs are seeded in large number, they will form a monolayer network, which later will become confluent.

Visualization of cell surface makers protein expression

Surface marker staining using fluorescent dye tagged antibody can be used to visualize the surface markers on the cells using fluorescent microscopy. Visualization may apply either on single cell in suspension or in cells that are located in between of other cells in a certain tissue. Visualization of surface markers is also available on light microscopy, but the specimen should be stained by immunocyto- or immunohisto-chemistry method.[24] Figure 7 shows immunohisto- and immunocyto- chemistry staining of CD34 on endothelial cell membrane in paraffin section compared to cultured cells that were detached to make a spot specimen. In addition, visualization by immunohisto-chemistry is useful to observed protein expression such as collagen type-1 and osteocalcin by differentiating mesenchymal stem cells.[25]

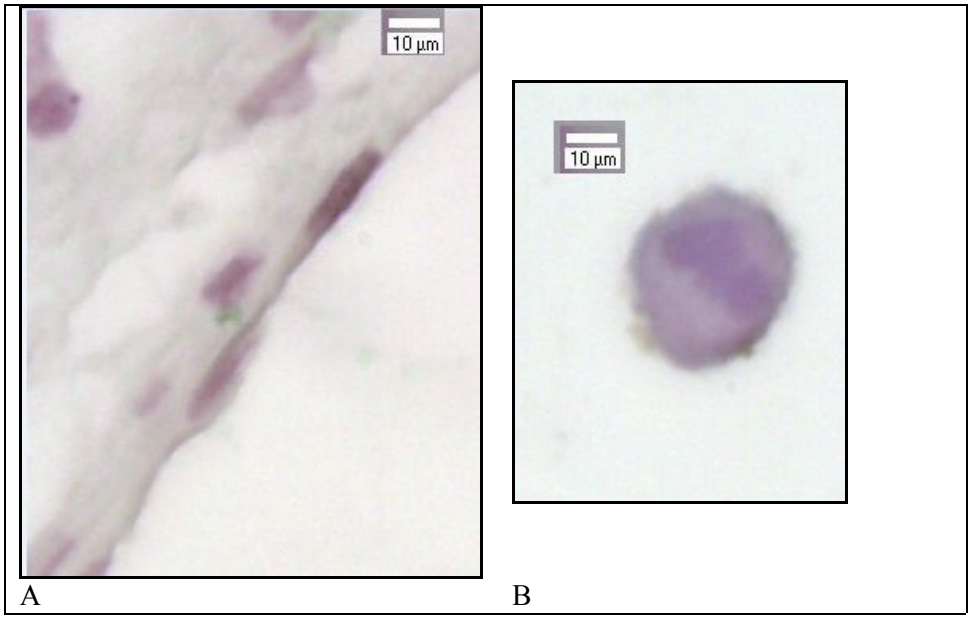

Figure 8. CD34 immunocyto-chemistry staining (light microscopy and Optilab digital camera)
A= a paraffin section shows CD34 positive endothelial cells, B= spot specimen shows a CD34 positive lipoaspirate derived MSCs

Visualization of cell differentiation

To prove differentiation capacity, the stem cells are induced using special medium and the result can be seen by various modes, and one of the modes are by staining either the cells or matrix that is produced by the cells. Especially for MSCs, differentiation into adipocytes can be visualized using a staining method i.e. by oil red O staining to visualize the lipid droplets inside the cells. Further, differentiation into chondrocytes and osteocytes can be detected by low pH alcian blue[26] and alizarin red[26, 27] or von Kossa staining to visualize cartilage matrix and calcification, respectively (Figure 8).[26] Differentiations into chondrocytes[28, 29] and adipocytes in adipose derived MSCs can also be achieved by prolonged culture.[29]

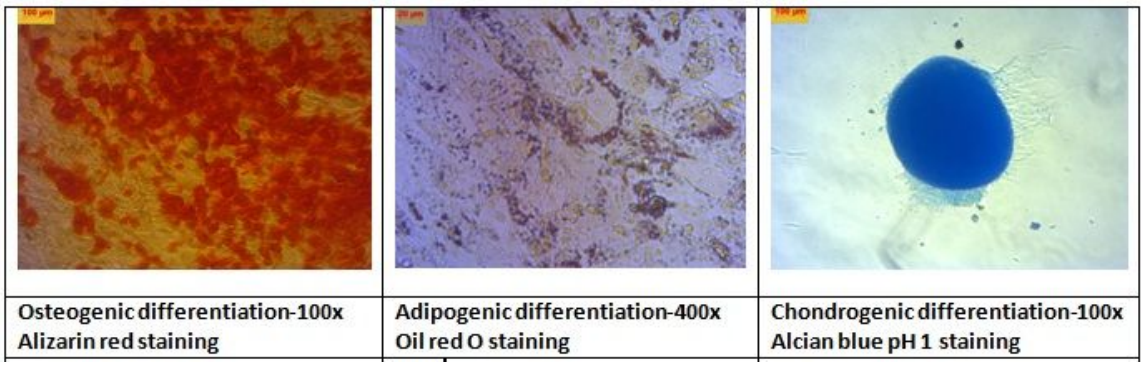

Figure 9. Umbilical cord derived MSC differentiation into three lineages (Nikon inverted microscope and Axiocam digital microscope camera) [18]

Visualization of cell senescence

Adult stem cells have limited capacity of cumulative doublings and will achieve replicative senescence after a certain number of cumulative doublings. Senescent cells are not appropriate to be used in regenerative medicine, and therefore the cells should be ascertained that they have not undergone senescence. Senescent cells produce β galactosidase, which activity can be visualized by cytological staining. Moreover, aging cells tend to be larger in size (Figure 10). [13]

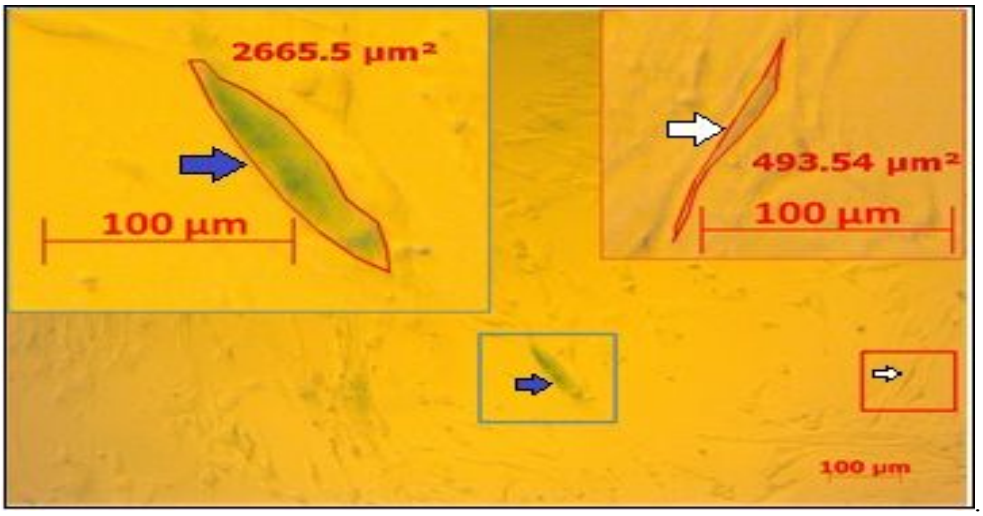

Figure 10. Senescent staining of umbilical cord derived MSCs (Nikon inverted microscope and Axiocam digital microscope camera) [13]

Conclusion

Visualization plays a substantial role in characterization and assuring the quality of stem cells.

Acknowledgement

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References

1. Pawitan JA. Prospect of adipose tissue derived stem cells in regenerative medicine. Cell &tissue transplantation &therapy 2009, 2, 7-9.

2. Mohamadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, Bagheri M, Bashtar M, Ghanaati H, Baharvand H, Ghavamzadeh A , Malekzadeh R. Phase 1 Trial of Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Decompensated Liver Cirrhosis. Archives of Iranian Medicine 2007, 10 (4), 459 –466.
3. Wang P, Li Y, Huang L, Yang J, Yang R, Deng W, Liang B, Dai L, Meng Q, Gao L, Chen X, Shen J, Tang Y, Zhang X, Hou J, Ye J, Chen K, Cai Z, Wu Y, Shen H. Effects and Safety of Allogenic Mesenchymal Stem Cell Intravenous Infusion in Active Ankylosing Spondylitis Patients Who Failed NSAIDs: A 20-Week Clinical Trial. Cell Transplantation 2014, 23, 1293–1303.
4. Jo CH, Lee YG, Shin WH, Kim H, Chai JW, Jeong EC, Kim JE, Shim H, Shin JS, Shin IS, Ra JC, Oh S, Yoon KS. Intra-Articular Injection of Mesenchymal Stem Cells for the Treatment of Osteoarthritis of the Knee: A Proof-of-Concept Clinical Trial. Stem Cells 2014, 32, 1254–1266.
5. Swain RP, Subudhi BB, Mahapatra AK, Bolapareddi V. Bridging Between Disease, Prevalence and Treatment of Diabetes Mellitus : A Review. International Journal of PharmTech Research 2014-2015, 7(2), 212-228.
6. Shabeenataj S, Priya L. Stem Cell Therapy for Thalassemia: A Review. International Journal of PharmTech Research; 2014, 6 (4), 1306-1308.
7. Bhowmik D, Chiranjib,Chandira RM,Tripathi KK, Sampath Kumar KP. Nanomedicine-An Overview. International Journal of PharmTech Research 2010, 2 (4), 2143-2151.
8. Harichandan A, Hans-Jörg Bühring H-J. Prospective isolation of human MSC. Best Practice & Research Clinical Haematology 2011, 24 , 25–36.
9. Ali H, Al-Mulla F. Defining umbilical cord blood stem cells. Stem Cell Discovery 2012, 2 , 15-23.
10. Snyder EY, Teng YD. Stem Cells and Spinal Cord Repair. N Engl J Med 2012, 366 (20),1940-1942.
11. Moody SA, Klein SL, Karpinski BA, Maynard TM, LaMantia A-S. On becoming neural: what the embryo can tell us about differentiating neural stem cells. Am J Stem Cells 2013, 2(2), 74-94.
12. Pawitan JA, Damayanti L, Bustami A, Swantari NM. Detection of Morphological Changes in Adipose Tissue Derived Stem Cells after Passage by the Simple Spot Method. J US-China med Sci 2011, 8(2), 92-98.
13. Mediana D, Liem IK, Pawitan JA, Goei N.Passage Effect on Aging of Human Umbilical Cord Derived Mesenchymal Stem Cell. Online J Biol Sci 2015, 15(3), 170-177.
14. Sudiarta KE, Satuman, Riawan W, Muliartha KG, Mintaroem K, Aulanni'am A, Ali M. The Efficacy of Taraxacum officinale Leaves Extract in Regulate Apoptosis, RARß2 gene and Sox2 expression on Primary Culture Human Cervical Cancer Stem Cells. International Journal of PharmTech Research 2015, 8(4), 535-544.
15. Pawitan JA, Liem IK, Budiyanti E, Fasha I, Feroniasanti L, Jamaan T, Sumapradja K. Umbilical cord derived stem cell culture: multiple-harvest explant method. International Journal of PharmTech Research 2014, 6(4), 1202-1208.
16. Rao M, Gauthami R, Saranya D, Prashanth Kumar HP. Regenerative Medicine- Scaffolds. International Journal of PharmTech Research; 2015, 8(5), 183-191.
17. Isparnadi E, Hidayat M, Aulanni'am A, Permatasari N. Characterization and Formulation of the Bivalve Anodonta's Chitosan-Platelet Rich Plasma-Mesenchymal Stem Cells as a Composite Scaffold. International Journal of PharmTech Research; 2015, 8(6), 718-724.
18. Pawitan JA. Future research in adipose stem cell engineering. In: Yves-Gerard Illouz, Aris Sterodimas, editors. Adipose stem cells and Regenerative Medicine. Heidelberg; Springer: 2011. p 257-272.
19. Pawitan JA, Damayanti L, Bustami A, Swantari NM. Detection of Morphological Changes in Adipose Tissue Derived Stem Cells after Passage by the Simple Spot Method. J US-China med Sci 2011, 8(2), 92-98.
20. Dominici M, Blanc KL, Mueller I, Cortenbach IS, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement Cytotherapy 2006, 8(4), 315-317.
21. Tan KY, Teo KL, Lim JFY, Chen AKL, Reuvevy S, Oh SKW. Serum-free media formulations are cell line specific and require optimization for microcarrier culture. Cytotherapy 2015, 17, 1152-1165.
22. Jing D, Fonseca A-V, Alakel N, Fierro FA, Muller K, Bornhauser M, Ehninger G, Corbeil D, Ordemann R. Hematopoietic stem cells in co-culture with mesenchymal stromal cells - modeling the niche compartments in vitro. Haematologica 2010, 95, 542-550.

23. Jurgens WJFM, Oedayrajsingh-Varma MJ, Helder MN, Doulabi BZ, Schouten TE, Kuik DJ, Ritt MJPF, van Milligen FJ. Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: implications for cell-based therapies. Cell Tissue Res 2008, 332, 415–426.
24. Pawitan JA, Wulandari D, Suryani D, Damayanti L, Liem IK. Comparison of flowcytometric and immunocytochemistry analysis of stem cell surface markers. Online J Biol Sci 2015, 15 (1), 1-5.
25. Isparnadi E, Hidayat M, Aulanni'am A, Permatasari N. Study of osteocalcin and collagen type I in Regeneration of Atrophic Non-Union Fracture based on Bivalve Anodonta-PRP Formula and Mesenchymal Stem Cells as Composite Scaffold in Regeneration of Atrophic Non-Union Fracture. International Journal of PharmTech Research; 2015, 8(3), 1041-1046.
26. Pawitan JA, Kispa T, Mediana D, Goei N, Fasha I, Liem IK, Wulandari D. Simple production method of umbilical cord derived mesenchymal stem cell using xeno-free materials for translational research. J Chem Pharm Res 2015, 7(8), 652-656.
27. Dilogo IH, Kholinne E. The Effect of Granulocyte Colony Stimulating Factor Administration on Mobilization, Proliferation and Differentiation of Mesenchymal Stem Cells. International Journal of PharmTech Research; 2015, 8(10),180-189.
28. Pawitan JA, Feroniasanti L, Kispa T, Dilogo IH, Fasha I , Kurniawati T, Liem IK. Simple method to isolate mesenchymal stem cells from bone marrow using xeno-free material: a preliminary study. International Journal of PharmTech Research; 2014-2015, 7(2), 354-359.
29. Pawitan JA, Suryani D, Wulandari D, Damayanti L, Liem IK, Purwoko RY. Prolonged culture in FBS and FBS-substitue containing media: Spontaneous chondrogenic differentiation of adipose tissue derived mesenchymal stem cells. International Journal of PharmTech Research; 2014, 6(1), 224-235.

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