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# Comparison Of Biological Characteristic And Osteogenic Differentiation Between Bone Marrow And Adipose Mesenchymal Stem Cell In Various Age Group

**William Chandra*, Ismail Hadisoebroto Dilogo**

**Department of Orthopaedic &Traumatology, Cipto Mangunkusumo Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.**

**Abstract : Introduction.** Mesenchymal stem cell is the answer of complicated medicine problems, including orthopaedic. Bone marrow is still the main source. Because of limited source, invasive procedure, pain, and relative less cell, adipose will be a promising source with equal regenerating and differentiating ability. Along with increasing life expectancy, geriatric population is increasing as well as the potential need for stem cell application. Yet there is still controversy about stem cell quality in aging.

**Methods.**This study was conducted in Stem Cell Medical Technology Integrated Service Unit Cipto Mangunkusumo Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, October 2015 - March 2016. 12 patients were divided into 3 age group; 15-30 year, 31-40 year, and 41-55 year. Bone marrow from posterior iliac crest and adipose tissue were collected, mesenchymal stem cell isolation and culture were done subsequently. Biological characterization, Population Doubling Time, osteogenic differentiation, and alizarin red assaywere carried out. All data was analyzed using SPSS 20.

**Results.** No significant difference was observed in biological characteristic and osteogenic differentiation of bone marrow and adipose mesenchymal stem cell among age group ($p>0.05$). There is significant difference in Population Doubling time in31-40 year group($p=0.000$) and 41-55 year group($p=0.000$).

**Conclusions**. Adipose mesenchymal stem cell had steady biological characteristic, Population Doubling Time, and osteogenic differentiation. Bone marrow mesenchymal stem cell had increasing population doubling time in increasing age, apart from biological characteristic and osteogenic differentiation. Adipose could be the source of choice in harvesting mesenchymal stem cell at any age.

**Keywords**: Mesenchymal Stem Cell,Osteogenic Differentiation, Bone Marrow, Adipose, Age.

## Introduction

Mesenchymal stem cell has self regenerating, rapid proliferating, and multipotent capability.[1,2]Bone marrow was the main source.Limited source, relative less cell, and invasive procedure aggravated exploration of other source. [3,4]With equal regenerating and differentiating capability, adipose tissue recently rised up as another source. Furthermore, it had abundant resources, more amount of cells, and relative less invasive harvesting procedure.[4-6]

Commonly, research was stressed on applying stem cell in curing disease but currently there was no direct study comparing adipose and bone marrowderived mesenchymal stem cells from one individual source.[7]Increasing human life expectancy createselderlypeoplewho prefer their own autogenous stem cell, and automatically raise the question about aging in mesenchymal stem cell.[3,8,9]Some studies had been carried out dan gave various result. Indeed, there is need to do comparative study of characterization and osteogenic differentiation of bone marrow and adipose mesenchymal stem cells in various age group.

## Experimental

This observational analytical in vitro study was conducted in Stem Cell Medical Technology Integrated Service Unit Cipto Mangunkusumo Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, October 2015-March 2016. 6patients were divided into 3 age group; group 1 (15-30 year), group 2 (31-40 year), and group 3 (41-55 year). This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia. Bone marrow aspiration from posterior illiac crest and adipose tissue harvest (hip, elbow, and back) were carried out in every subject. Mesenchymal stem cell isolation and culture were done afterwards.

Bone marrow was cultured in medium of 100U Penicilin/100 µg Streptomycin/ml, 0.25 µg Fungizone/ml, 10U heparin, 1% Glutamax, 10% PRP, and alpha minimum essential medium (α-MEM). The samples were then centrifugated and cultured on media with temperature $37^0$C, 5%$CO_2$ and 20% $O_2$ until there was confluence. Meanwhile, adipose tissues were cut into small pieces, type I collagenase was added. After centrifugation, the samples were cultured in medium of 1% Amphotericin B and 10% PBS with temperature $37^0$C, 5%$CO_2$ and 20% $O_2$ until there was confluence. After reaching confluence on the first passage, mesenchymal stem cell were cryopreserved.on MVE 800 Series Cryo Tank with temperature -$190^0$C.

After thawing, subculture were performed until passage 3. Population Doubling Time (PDT) and Flowcitometry analysis were taken part. PDT was calculated with formula t/n; n = log(Nh) – log(Ni)/ log2, where Nh: total cell count at harvest, Ni: total cell at first, t: duration of culture, and n: population doubling time. In Flowcytometry analysis, samples were then incubated with fluorescein isothiocyanate (FITC) that conjugated with anti-monoclonal CD34, CD73, CD 105, and CD90. Cells were analyzed using BD FACSCalibur flowcytometer (BD Biosciences).

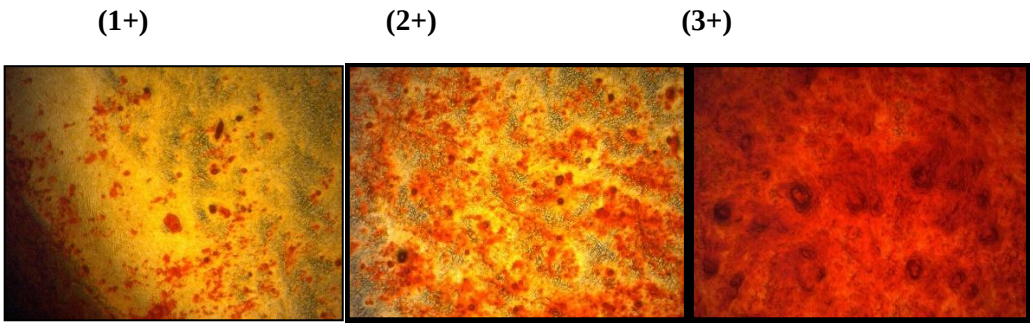



**Figure 1. Alizarin Red assay assessment criteria.**

Osteogenic differentiation was carried out in 8 mL DMEM, using StemPro® Osteogenesis Differentiation Kit (Gibco)in $37^0$C, 20% $O_2$, 5% $CO_2$ for 21 days.Alizarin Red Assay was performed. Cell count was done in 5 high power field (HPF) for 2 times by author, and was interprated as such: (1+) if $<^1/_3$ HPF was enhanced; (2+) if $^1/_3$-$^2/_3$ hpf was enhanced; and (3+) if $>^2/_3$ hpf was enhanced (figure 1). Data analysis was done using Statistical Product and Service Solution (SPSS) version 20.0 program.

## Results

The mean age of subjects was 34,92 ± 8.15 years old. Most of the subjects were male (83,33%) and harvested from the hip adipose. Characterization result of surface biomarker CD34, CD73, CD90, and CD105showed no significant differences among various age groups. ($p>0.05$, 95%CI) (figure 2).

Population Doubling Time as evaluation of growth pace was conducted. Mann-Whitney Test showed significant difference on group 2 (31-40 year old)($p<0.05$, 95%CI) and group 3 (41-55 year old) ($p<0.05$, 95%CI) as shown in Figure 3.Population Doubling Time was also analyzed based on the origin of source, bone marrow and adipose tissue. On bone marrow derived mesenchymal stem cells, post hoc analysis with Bonferroni multiple comparisons showed significant difference between group 1 (15-30 year old) and group 3 (41-55 year old) ($p=0.048$, 95%CI). On adipose tissue, one-way Anova test showed no significant difference among the age group ($P>0.05$, 95%CI) as shown in Figure 4.

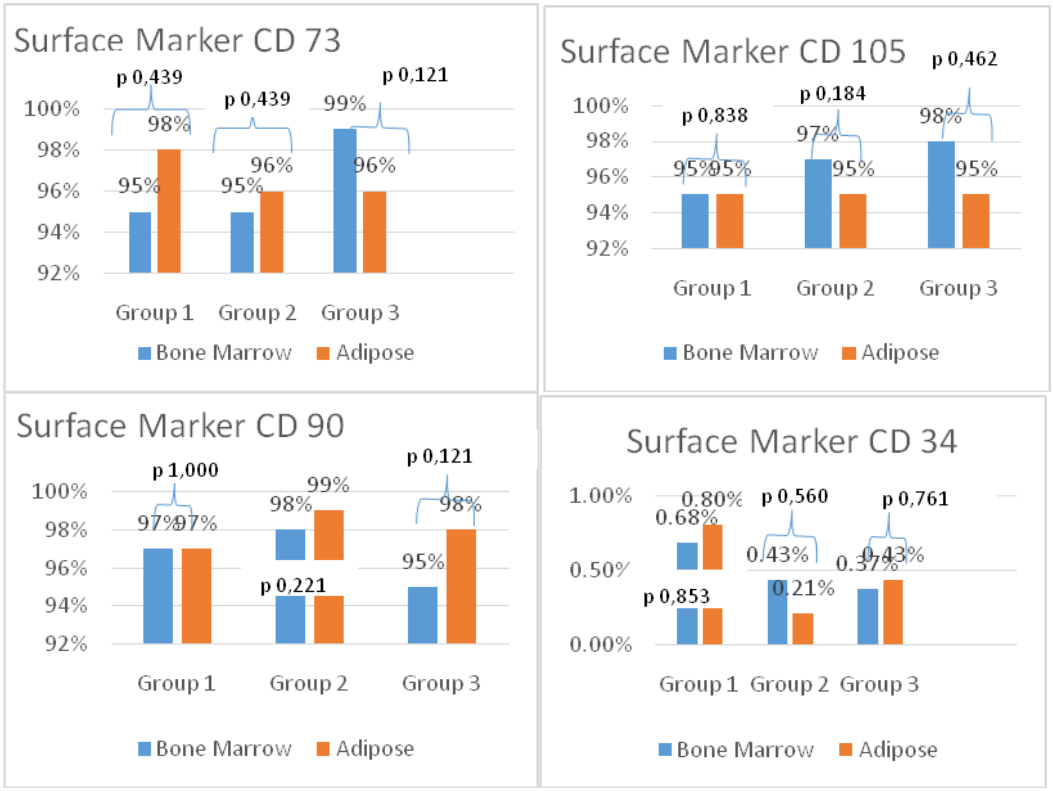



**Figure 2. Surface Marker CD 73, CD 105, CD 90, CD 34 evaluation**

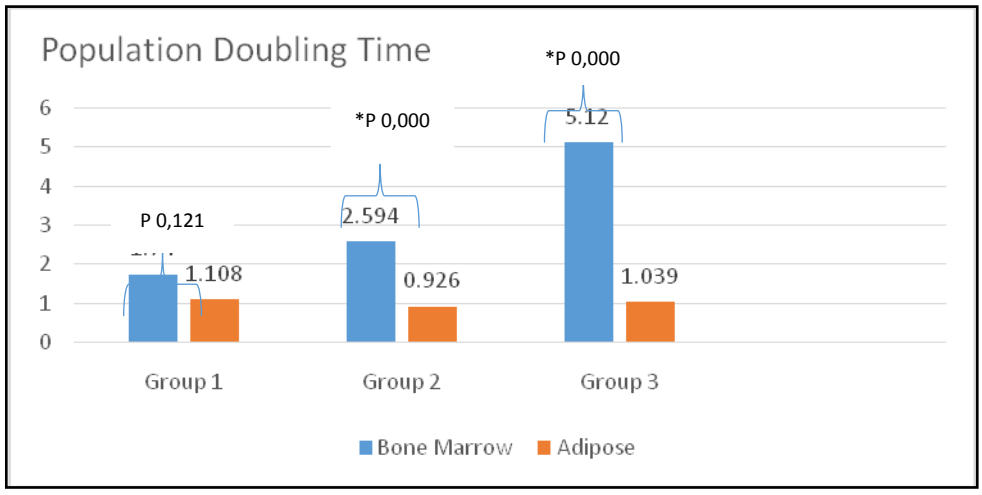



**Figure 3. Population Doubling Time evaluation in various age group**

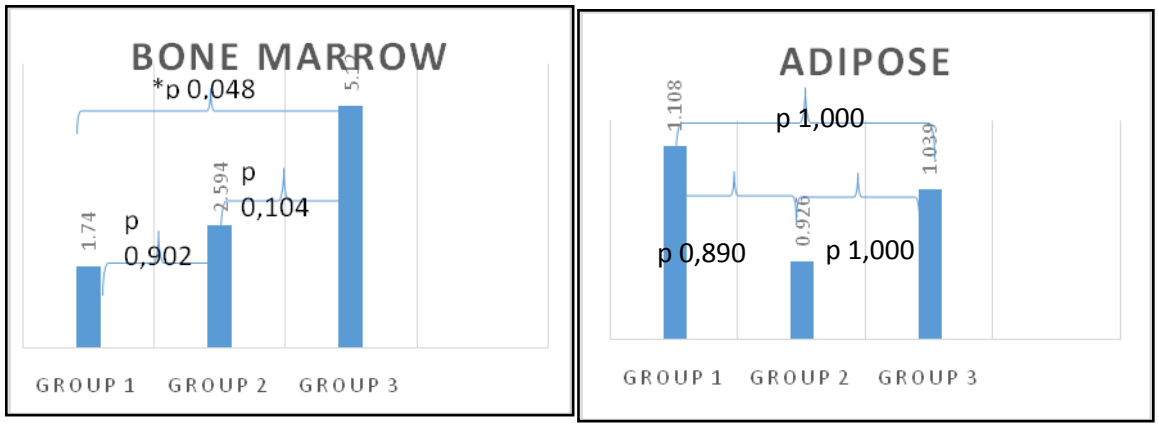
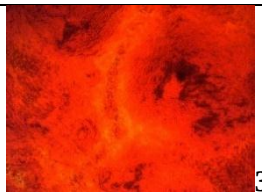
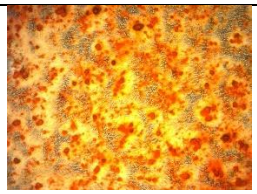
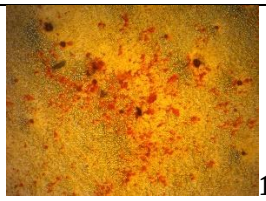
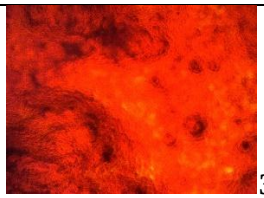
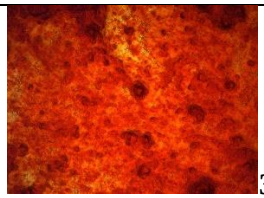



**Figure 4. Population Doubling Time evaluation in various sources**

Alizarin Red assay was performed to evaluate extra cellular calcification which signified osteogenic differentiation. Kruskal-Wallis test showed no significant difference was found in osteogenic differentiation among various age groups. (p>0.05, 95%CI) (Table 1).

**Table 1. Alizarin Red assay evaluation in various age group**

| | **Group 1** | **Group 2** | **Group 3** |
|---|---|---|---|
| Bone Marrow |  **3+** |  **2+** |  **1+** |
| Adipose |  **3+** |  **3+** |  **3+** |
| P value | 1,000 | 0,083 | 0,083 |

## Discussion

This study was an observational analytical in vitro methods with 12 samples of mesenchymal stem cells derived from bone marrow and adipose tissue in various age groups. Subjects was divided into three age groups: 15-30 years old, 31-40 years old, and 41-55 years old. Stolzing et al stated that in>40 years old people, bone marrow derived mesenchymal stem cell had decreased proliferating and differentiating potency.[10] Another studies stated that PDT on adipose derived mesenchymal stem cell was significantly different at >40 years old[11] and the amount of apoptoses increased by age.[12]

Bone marrow was selected due to the widely accepted and gold standard source. Indeed, it had several weaknesses, including less number of cell and declining proliferating and differentiating ability by age.[1,7] Because of abundant sources, less invasive procedure, and relative more cell, adipose will be a promising source with equal regenerating and differentiating ability.[13]

All of the subjects showed homogenous distribution in the range of 19-44 years old. Bone marrow was harvested from posterior illiac crest which was thought to have the largest amount of bone marrow and reproducible result. While the location of the adipose tissue derived largely from the hip. Subjects were mainly man to minimize decreasing bone marrow differentiation capability in perimenopause age.

All MSC samples was cryopreservated at passage 1. This was conducted because bone marrow and adipose tissue samples were obtained in different time over a period of 3 months.Furthermore, cryopreservation was aimed to standardized the process for each sample. Minonzio et al showed that cryopreservation until 193 days didn't changed the amount, characteristic, viability, and proliferation potency of MSC.[14]

Flowcytometry was carried out to compare the surface marker CD 73, CD 105, CD 90 and CD 34 on the samples at passage 3. All samples had fulfilled minimal criteria of MSC[7,15] anddid not have significant difference among age group. This was equal with Choudhery et al that stated the age did not affect the difference of morphology and phenotype of MSC from adipose tissue and Labora et al that stated there was no significant difference in MSC surface marker by age.[8,16]

PDT of bone marrow and adipose MSCs was compared among age group. In31-40 year old group and 41-55 year old group, bone marrow and adipose tissue derived MSC was significantly different. This was equal with Choudhery et al, Schipper et al, and Labora et al that showed the age did not affect proliferation rate of adipose MSC, in contrast to bone marrow.[8,12,16] This study showed a significant decrease in proliferation rate of MSC derived bone marrow along with age,similar to Nakahara et al.[17] Another study showed Vincolin gene that was expressed on low quantities at a young age and increasing with age also affect the rate of proliferation.[16] Bujalska et al showed that the enzyme activity of hydroxysteroid 11β-dehydrogenase type 1 (11β-HSD1) was responsible for the decline in the proliferation on elderly that the enzyme had a role in producing glucocorticoid in the cell. Transforming growth factor β1 (TGF- β1) induced aging on MSC derived bone marrow by increasing production of reactive oxygen species (ROS) from mitochondria, resulting in a decrease in mitochondrial membrane potential, the destruction of DNA, and cell aging.[10,18] This was explained Labora et al where TGF- β1 and ROS increased significantly in elderly.[16]This study showed that in elderly, MSCs are better harvested from adipose tissue that has a PDT constantly in every age.

All samples showed enhancement in Alizarin Red assay, so osteogenic differentiation was confirmed. Author created a semi quantitative assessment of osteogenic differentiation in Alizarin Red assay. This assessment showed no significant difference of extracellular calcification among age group. There was roughly a difference in enhancement but it was not statistically different. Zhu et al stated there age did not significantly affect osteogenesis in adipose tissue derived MSC but Choudhery et al found a contradiction. It was still a debatable thing, and will further need another osteogenic marker. Estrogen was thought to influence the osteogenic differentiation in elderly. In this study, there was no woman subject in perimenopausal age so it was neglectable.

## Conclusion

There was no difference on biological characteristic of bone marrow and adipose tissue MSC in every age group. There was significant difference in PDTbetween bone marrow and adipose MSC in 31-40 year old and 41-55 year old group. No difference in osteogenic differentiation and Alizarin Red assay was found between bone marrow and adipose MSC in each age group. Adipose MSC had steady biological characteristic, osteogenic differentiation, and Population Doubling Time in each age group. Meanwhile, bone marrow MSC had increasing population doubling time in increasing age, apart from biological characteristicand osteogenic differentiation that constant at any age. Adipose could be the source of choice in harvesting mesenchymal stem cell at any age.

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