APPENDIX

Determination of PBSCs potency in terms of cell kinetics, growth and differentiation in culture system: effects of age and sex of donors

Nutthapong Moonkum, Jiraporn Kantapan and Samlee Mankhetkorn*

Laboratory of Physical Chemistry, Molecular and Cellular Biology; Center of Excellence for Molecular Imaging, Department of Radiologic Technology, Faculty of Associated medical sciences, Chiang Mai University, Chiang Mai 50200 Thailand

***Corresponding author:**

Samlee Mankhetkorn, Ph.D.

Laboratory of Physical Chemistry, Molecular and Cellular Biology; Center of Excellence for Molecular Imaging, Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200 Thailand

Tel. 6653949305 Fax. 6653213218

e-mail: samlee@cemithai.com/samlee.mankhetkorn@cmu.ac.th

Abstract

The biological and potency of PBSCs obtained from the PBMCs fraction of 12 healthy donors including both male and female who have adult and elderly age and have blood group O. The PBMCs obtained by the ficoll-centrifugation were not significantly difference and numbers ofanti-CD34+-FITC positive staining cells were about 1.2 ± 0.2 %. The PBSCs composed of two subpopulations, the mesenchymal and the hematopoietic stem cells. Once they were cultured in the same conditions as monolayer and 3D-culture systems, exhibited similar potency in terms of growth and differentiation independently on sex, and age of the donors. The cells have homeostasis properties and were strictly controlled the balance of cell numbers. The kinetic parameters determined for the 12 donors including the mean rate of cell to re-entering to the cell cycle (γ), move from S to G2/M (k2), move from G2/M to G0/G1 (k1) undergone apoptosis (β) and differentiation (α) was equal to 0.69 ± 0.51 cell.h^{-1}, 0.17 ± 0.28 cell.h^{-1}, 0.11 ± 0.13 cell.h^{-1}, 0.05 ± 0.06 cell.h^{-1}, 0.64 ± 0.49 cell.h^{-1},respectively. This is the first results from small group of donors, that certainly cannot represent all normal subjects, but crucial for determining the parameters indicating the status of normal cells.

Keywords: Peripheral blood stem cells (PBSCs), mesenchymal stem cell, hematopoietic stem cell, ficoll-hypaque, homeostasis property, 3D-nanofibrous PVDF scaffold

Introduction

Peripheral blood is a large accessible source of adult stem cells. Especially, the blood mononuclear fraction obtained by gradient-centrifugation has been studied for more than 50 years. This fraction contains a multitude of distinct multipotent cells that possess the potential to differentiate in almost cell types during culture in specific condition medium including blood cells, endothelial cells [1], hepatocytes [2], cardiomyogenic cells [3], muscle cells [4], osteoclasts [5], osteoblasts [6], epithelial cells [7], neural cells [8] and myofibroblasts [9]. Recent researches showed that there were very small amounts of stem cells (0.1% of leucocytes [10]) contain in the PBMCs fraction the so-called peripheral blood stem cells (PBSCs) that should responsible to the growth and differentiation of the PBMCs fraction. Up to date, PBSCs can be maintained and expanded in cell culture systems and were demonstrated to be composed of two subgroups including hematopoietic and mesenchymal stem cells. However, the potency and growth efficiency of the cells depended on the conditions of selection, isolation and culture conditions [11].

In human body, PBSCs are regarded as readily available reservoirs of reparative cells able to mobilize, proliferate and differentiate to the appropriate cell type in response to specific signals [12]. In addition the stem cells have indispensable regenerative, reparative, angiogenic and immunosuppressive properties that point out their application in biomedical research and therapeutic potential. PBSCs may be the superior to other sources in cell-based therapy for myocardial regeneration [13]. Unfortunately their potential use, the variables influencing the potency of PBSCs still need to be elucidated. The influenced parameters such as aging [14], gender [15] and microenvironment in cell culture [16] were demonstrated to be key factors of the potency of PBSCs effect on the behavior and morphology of the PBSCs. However, it was very difficult to compare the efficiency and capacity of the stem cells of a different sources reported in international literature due to the different methods of isolation and expansion used of each research group.

Therefore, the study rigorously isolated the PBSCs from 12 healthy donors without any pretreatment by mobilization protocol. The PBSCs were obtained using the

ficoll gradient centrifugation technique and were cultured both in conventional and 3D-culture system. The results of the study allowed determining the parameters of cells kinetics such as the mean rate of cell to re-entering to the cell cycle (γ), move from S to G2/M (k2), move from G2/M to G0/G1 (k1) undergone apoptosis (β) and differentiation (α). It was well accepted that stem cells need 3D-extracellular matrix for the differentiation and regeneration to a given specialized cells and tissue [17]. Thus 3D-nanofibrous PVDF scaffold were used as their extracellular matrix.

The results demonstrated that the PBSCs isolated from all donors were not significantly difference (P = 0.44). Once they were cultured in the same conditions, such as in RPMI 1640 completed with 10% fetal bovine serum and 1% penicillin-streptomycin as monolayer and 3D-culture systems, exhibited similar potency in terms of growth and differentiation independently on sex, and age of the donors. The cells have homeostasis properties and were strictly controlled the balance of cell numbers by which can be used as a characteristic of normal PBSCs.

Materials and Methods

PBSCs donors

The project was approved from the Human Research Ethics Committee of Faculty of Associated Medical Sciences, Chiang Mai University (ref. no.361/2556).

Twelve healthy donors who have blood group O were recruited and inclusion into the study was based on a questionnaire. The stem cell donors were divided in two age groups. First group was the adult age that comprised male and female who have age between 15 to 40 years and the other was elderly age that also comprise male and female with age between 50 to 70 years.

Collection of human peripheral blood mononucleated cells (PBMCs) from donors

Whole bloods (100 mL) were collected in the presence of Heparinized (1430 USP units) after the donors agreed and signed the consent form. Whole bloods were centrifuged at 1500 rpm for 30 min and the buffy coat was separated. The total volume of buffy coat 4 mL was transferred to a new 15 mL sterile tube then completed with 4

mL modified-DPBS (MD-DPBS) and gentle mixed. The Ficoll-hypauqe (4 mL) was carefully injected at the bottom of the tube prior to centrifugation at 1500 rpm for 30 min. The PBMCs that consists the PBSCs were isolated and washed once using sterile MD-DPBS. The PBMCs fraction was twice re-suspended in RBC lysing solution for 5 minutes. After that the cells were washed twice using sterile MD-DPBS then were resuspended in RPMI1640 w/o L-glutamine and phenol red, supplemented with 10 % human serum group O or fetal bovine serum and 1 % penicillin /streptomycin (BioMedia) and placed at 37 °C in 5 % CO_2 and 95%humidity in an incubator.

Enumeration of PBMCs and $CD34^+$ cells

The $CD34^+$ cell counts were performed by anti-$CD34^+$-FITC staining and analyzed by flow cytometer. Briefly, cells (10^6) were centrifuged at 7,000 rpm for 1 minute and washed once using MD-DPBS pH 7.4 at 25°C. Afterward, 1 mL ice-cold ethanol (70% v/v) was added drop wise, with gentle agitation and further incubation at 4 °C overnight. The cells were centrifuged at 7,000 rpm for 1 minute. The pellets of cells were discarded then 5 µl Triton x-100 (0.1% v/v), 50 µl RNase (0.2 mg/mL) and 10 µl anti-$CD34^+$-FITC was added prior to further incubation in the dark at room temperature for 30 minutes. Add 440 µl MD-DPBS vortex briefly, analyzed in a flow cytometer and fluorescence microscope (Leica, Germany).

Flow cytometric analysis of cellular DNA contents

Cells (3×10^5) were centrifuged at 7,000 rpm for 1 minute and washed once using MD-DPBS pH 7.4 at 25°C. Afterward, 1 mL ice-cold ethanol (70% v/v) was added drop wise, with gentle agitation and further incubation at 4 °C overnight. The cells were centrifuged at 7,000 rpm for 1 minute. The pellets of cells were discarded then 5 µl Triton x-100 (0.1% v/v), 50 µl RNase (0.2 mg/mL) and 5 µL propidium iodide (1 mg/ml; US biological, USA) were added prior to further incubation in the dark at room temperature for 30 minutes. Add 440 µl MD-DPBS vortex briefly, analyzed in a flow cytometer and fluorescence microscope (Leica, Germany).

H&E staining

H&E staining was performed for the adherent cell fraction. Briefly, the medium and suspension cells were removed and the cells were washed twice using modified-DPBS pH 7.4 at 25°C. The cells were fixed on the culture plates using 500µL formalin (4% v/v) and let incubated at room temperature for 15minutes then washed once using MD-DPBS. The membranes of cells were permeabilized using 500 mL Triton-x100 (0.1% v/v) at room temperature for 10 minutes. The cells were washed with MD-DPBS following a dehydration process by adding 500 µL of ethanol with sequential incremental percentage of 70%, 95%, and absolute ethanol. Afterward, 500 mL hymatoxylin was added and placed at room temperature for 30 minutes, washed once using tab water. Add 500 µl ethanol (80% v/v) containing 1% HCl. Add 500 µl eosin and incubated at room temperature for 5 minutes and dehydration in 500 µl 70%, 95%, absolute ethanol. The cell morphology was observed under high resolution microscope.

Expansion of adult PBSCs in conventional culture

Cells (10^6 cell/mL) were cultured in 24-well plates with RPMI-1640 w/o L-glutamine and phenol red supplemented with 10 % human serum group O or fetal bovine serum and 1 % penicillin /streptomycin (BioMedia) at 37 °C in 5 % $CO_{2(g)}$ atmosphere in a humidified incubator at 95% humidity. The cell morphology was examined under an inverted light microscope every 24 hours. The cells were counted using a flow cytometer.

PBSCs growth and differentiation on PVDF 3D-nanofibrous scaffold

The 3D-nanofibrous PVDF scaffolds were disinfected by purging in 70% alcohol for 30 minutes and were washed using sterile phosphate buffer pH 7.4 at room temperature in biohazard cabinet. The scaffolds were UV-C irradiated for 1 hr then immersing into sterile RPMI-1640 medium and let incubated at 37 °C for 24 hrs in a CO_2-incubator.The scaffolds were transferred to 6-well plates. PBMCs (60 µL of 10^7 cells) were seeded onto the scaffolds and further incubation at 37 °C for 24 hrs in a CO_2-incubator, then 4 mL of fresh RPMI 1640 medium was added into the wells and

further incubation for 72 hrs. Then the scaffolds were transferred into new 6-well plates and completed with 4 mL of RPMI 1640, incubated at 37 °C in a CO_2-incubator. The culture was maintained by changing the culture medium once a week.

Scanning electron microscopy sample preparation

The scaffolds were firstly fixed by immersing 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 for 1 hours at room temperature or at 4° C (in refrigerator) overnight. They were washed 2 times in 0.1 M cacodylate buffer pH 7.4; each 10 minute duration. Then the second fixation was performed by immersing the scaffolds in 1% osmium tetroxide (aqueous) pH 7.4 for 2 hour at room temperature and in a light tight container. The scaffolds were again washed 2 times in 0.1 M cacodylate buffer pH 7.4; each 10 minute duration. The scaffolds were dehydrated by sequentially immersing as follows: 1 x 10 min. in 30% ethanol, 1 x 10 min. in 50% ethanol, 1 x 10 min. in 70% ethanol, 1 x 10 min. in 80% ethanol, 1 x 10 min. in 90% ethanol, 1 x 10 min. in 100% ethanol. The scaffolds were then submitted to perform critical point dry which is an automated process takes approximately 40 minutes. The scaffolds were mounted onto metal stub with double sided carbon tape. Finally, a thin layer of gold and palladium were coated over the scaffolds using an automated sputter coater.

Results

Analysis of freshly isolated PBMCs from whole blood

PBMCs freshly isolated were spherical shape composed of two subpopulations as monocytes and lymphocytes. Figure 1 demonstrated that very small amounts of PBMCs were positively staining with anti-$CD34^+$-FITC. As clearly seen that the anti-CD34+-FITC positive staining cells were found in both suppopulations; 1.1% (Figure 1c) and 1.4% (Figure1d) were found in lymphocyte and monocyte fraction, respectively. Cellular DNA content of PBMCs was measured using propidium iodide (PI) staining and analyzed by flow cytometer. As indicated in Figure 2a, most of PBMCs were positively staining with PI. It should be noted that initially of PBMCs isolation the cells were found in different phases including G0/G1, S and G2/M (Figure

2b and c). The cellular DNA content was measured at every 24 hours after the PBMCs fraction was cultured.

The amounts of PBMCs isolated from 12 donors were indicated in Table 1. In our experimental conditions, the mean value of total PBMCs in male and female was equal to $3.7 \times 10^5 \pm 1.3 \times 10^5$, $3.6 \times 10^5 \pm 1.3 \times 10^5$cell/ml for adult, and $5.5 \times 10^5 \pm 2.7 \times 10^5$, $5 \times 10^5 \pm 1.72 \times 10^5$ cell/ml for elderly group, respectively. The result obtained from both groups was no-significantly difference (P= 0.28). In particularly, the anti-CD34+-FITC positive staining cell and the number of cell found in phase S and G2/M of male and female was equal to 8.64 ± 5.18, 3.29%for adult, and 2.81 ± 0.13, 2.11±1.77%for elderly with P-values equal to 0.31 signified that all subjects have similar amounts stem cells in blood.

The histogram of cellular DNA content was changed. For example, at day 6 an amount of cells were found in sub G0/G1 phase and the cells found in S and G2/M were increased (figure 2d).

Expansion of PBSCs in culture

1. Effects of different origin of serum on PBSCs culture

We have verified that the colony forming of stem cells determined in our system was dependence on the density of PBMCs; only the density of cells lower degree than 5×10^6 cell/mL can observe cell proliferation and colony forming units of the stem cells. The behavior of PBMCs when cultured using the medium completed with 10% serum of their own donors compared with the series using fetal bovine serum was indicated in Figure 3. It was clearly shown that afterward the PBMCs were suspended in the medium and placed in a CO_2-incubator for 24 hours, almost of cells were suspended and aggregated among them throughout the flask. Few of cells with spindle shape were found to attach on the bottom of the flask at day-6, however only few colonies were developed even after day-24, while the aggregation of cells were bigger proportionally to the incubation time. The attached cells at day 24 were majorly foam like cells (Figure 4). Contrary, the series using fetal bovine serum the PBSCs were found as single distributed throughout the culture flask. At day-6, the colonies of mesenchymal stem

cells were abundantly found attached onto the bottom of culture flask. When the cell cultures were prolonged to day 24, a variety of cell types were observed including, adipocytes, osteocytes, chondrocytes, neurons, leucocytes, stromal cells, etc (Figure 5).

2. Effects of sex and age on the potency of PBSCs culture

In order to compare the behavior of PBSCs obtained from adult and elderly age, the cells were cultured using the medium completed with 10% fetal bovine serum. Figure 6 showed that PBSCs grew and underwent differentiation similarly in all donors independently neither sex nor age. H&E staining of cells allowed identifying the specific cell type as indicated in Figure 5.

The potency of PBSCs growth and differentiation in the presence of biocompatible 3D-nanofibrous PVDF scaffold was indicated in figure 7. The PBSCs of all donors able to grow and undergo differentiation which yielding a tissue when they were cultured using the scaffold.

3. Analysis of cellular kinetics of PBSCs

On the microscopic analysis point of view, PBSCs composed of two subpopulations; one was adherent cell and the other is suspension cell. Both of cell types were founded the colony forming units of stem cells in the culture system but it was difficult to enumeration of the stem cells.

The study rigorously isolated only the suspension cells then the cells were analyzed by measuring their DNA content as a function of time. Figure 8a shows that these cells were found in different cell phases including G0/G1, S, G2/M and sub G0/G1 that corresponding to the apoptotic cells. The results of this study allow writing the diagram (Figure 8b) explaining the homeostasis which is the characteristic of normal stem cells. The parameters of cell kinetics including the mean rate of cell to re-entering to the cell cycle (γ), move from S to G2/M (k2), move from G2/M to G0/G1 (k1) undergone apoptosis (β) and differentiation (α) were determined as indicated in Table 2.

Discussion

Peripheral blood stem cells (PBSC) are increasingly used as an autologous source for cell therapies, including those for the regeneration of non-hematopoietic tissues including skeletal muscle [18], heart [19], and neurons [20]. The study was rigorously investigated the biological and potency of PBSCs obtained from 12 healthy donors including both male and female who have blood group O. The donors were divided into two groups; adult and elderly age.

It was clearly demonstrated that the PBMCs and the stem cell contents obtained from 12 donors were not significantly different (P = 0.24 for PBMCs and P = 0.31 for stem cells). This reflects that neither age nor sex influenced on the component of stem cells in healthy donors (at least for the 12 donors).

The results showed that PBMCs obtained from all donors exhibited the similar pattern of light scattering and were composed of lymphocytes and monocytes. And about 1% CD34+ staining cells were found in each population. When the cells were cultured conventional conditions with very high density of cells (> 10^7 cell/mL), the number of CD34+ staining cells increased as a function of incubation time and few of adherent cells were determined [10]. Interestingly, when the culture started with the density of cells < 5×10^6 cell/mL, it was found that the cells were behaved one part as suspension and the other part as adherent cells. The differentiation of PBSCs was clearly demonstrated by life cell imaging with optical light microscope and H&E staining (Figure 4 and 5). However, the differentiation cells were found to adhere on the culture flask or culture plate. For example in Figure 4, when the cells were cultured using culture medium completed with 10% of serum obtained from the same donors, the morphology of differentiation cells were found as keratinocytes, dendritic and foam cells which are kwon as the issue of hematopoietic stem cells. While the cultures were done using medium completed with fetal bovine serum a large varieties of differentiation cells were found including keratinocytes, lymphocytes, dentritic and foam cell like, adipocytes, osteocytes, chondrocytes, neurons, leucocytes and stromal cells, etc (Figure 5). As reported in international literature, the mesenchymal stem cells have an extensive proliferation capacity and they can differentiate into multiple lineages

namely, osteocytes, chondrocytes, adipocytes, astrocytes and myocytes. The HSCs can differentiate into leucocytes, neurons and cardiomyocytes [21]. These results strongly suggested that the PBSCs obtained with the conditions of experiments composed of both hematopoietic and mesenchymal stem cells. It should be noted that both hematopoietic and mesenchymal stem cells are always found as suspension cells, even they were cultured for long time period. In particularly, these mesenchymal stem cells were very sensitive to growth factors and chemokines. The particular finding was that in conditions of the experiments, the adherent cells that should corresponding to mesenchymal stem cells can be expanded in number to reach a confluence when were cultured with medium completed with 10% fetal bovine serum. It was well accepted that fetal bovine serum is more enrich of growth factors and chemokines than those from adult human serum [22].

The DNA content analysis of suspension cells showed that the majority of cells were found in G0/G1 phase equal to (80%) and the cells found in S and G2/M phase equal to (20 ± 3%) indicated that these 80% of cells were re-entering to the cell cycle, 10% of cells were differentiated to specialized cells and 10% of cells were undergo apoptosis. These results suggested that in the conditions of experiments, there was an existence of homeostasis that strictly control and balance the number of cells as illustrated in Figure 8b. In fact the homeostasis should be considered as one of the characteristic of normal stem cells [23]. Once the normal stem cells lost the homeostasis, the cells were completely changed and become cancer stem cells. Thus it is very important to determine the parameters of cell kinetics of the normal stem cells. These kinetic parameters determined for the 12 donors including the mean rate of cell to re-entering to the cell cycle (γ), move from S to G2/M (k2), move from G2/M to G0/G1 (k1) undergone apoptosis (β) and differentiation (α) was equal to 0.69 ± 0.51 cell.h^{-1}, 0.17 ± 0.28 cell.h^{-1} , 0.11 ± 0.13 cell.h^{-1}, 0.05 ± 0.06 cell.h^{-1}, 0.64 ± 0.49 cell.h^{-1}, respectively. This is the first results from small group of donors, that certainly cannot represent all normal subjects, but crucial for determining the parameter indicating the status of normal cells.

The results of this study also demonstrated that PBSCs can originate communities of cells and tissue as revealed by SEM micrographs (Figure 7). All PBSCs obtained in this study exhibited similar efficiency to yield communities of cells and tissue when were cultured 3D-nanofibrous PVDF scaffold. Contrary our expectation, the PBSCs obtained in this study exhibited similarly efficiency regeneration of communities of cells and tissues. It should be noted that the PBSCs were washed and be found in the same environment of culture, thus we can make a hypothesis that the PBSCs always preserve their own properties such as self renew and differentiation and this properties probably modulated by the chemical environment. Also when we encountered aging situation, this might be the chemical environment of our body found unsuitable for stem cell growth and regeneration (which is the repair mechanism of body).

The overall results of the study showed that it was possible to isolate PBSCs from whole blood healthy donor without any pretreatment using cytotoxic agents indicated in the mobilization protocol. These PBSCs were composed of both hematopoietic and mesenchymal stem cells that can maintain and expended in cell culture system. The PBSCs in conventional cell culture conditions can differentiate to varieties of specific cells including, adipocytes, osteocytes, chondrocytes, neurons, leucocytes, stromal cells, etc. In addition, the PBSCs efficiently regenerated new communities of cells and tissue in 3D-nanofibrous scaffold system. PBSCs exhibit homeostasis property which was proposed to be crucial biomarker of normal PBSCs. This study makes evidence that PBSCs is one of important source of stem cells which were very easy to achieve and expand in culture without any addition neither mitogens nor specific growth factors.

Acknowledgement: Kantapan J. got Ph.D. scholarship from the Ministry of Education (CHE-2551), Moonkum N and Kantapan J would to thank the Bureau of Graduate study, the Faculty of Associated Medical Sciences, Chiangmai University for partially financial support. This work was financially supported by a grant funded by The Strategic Scholarships for Frontier Research network for the Government, The higher Commission on Higher Education, Ministry of Education Thailand.

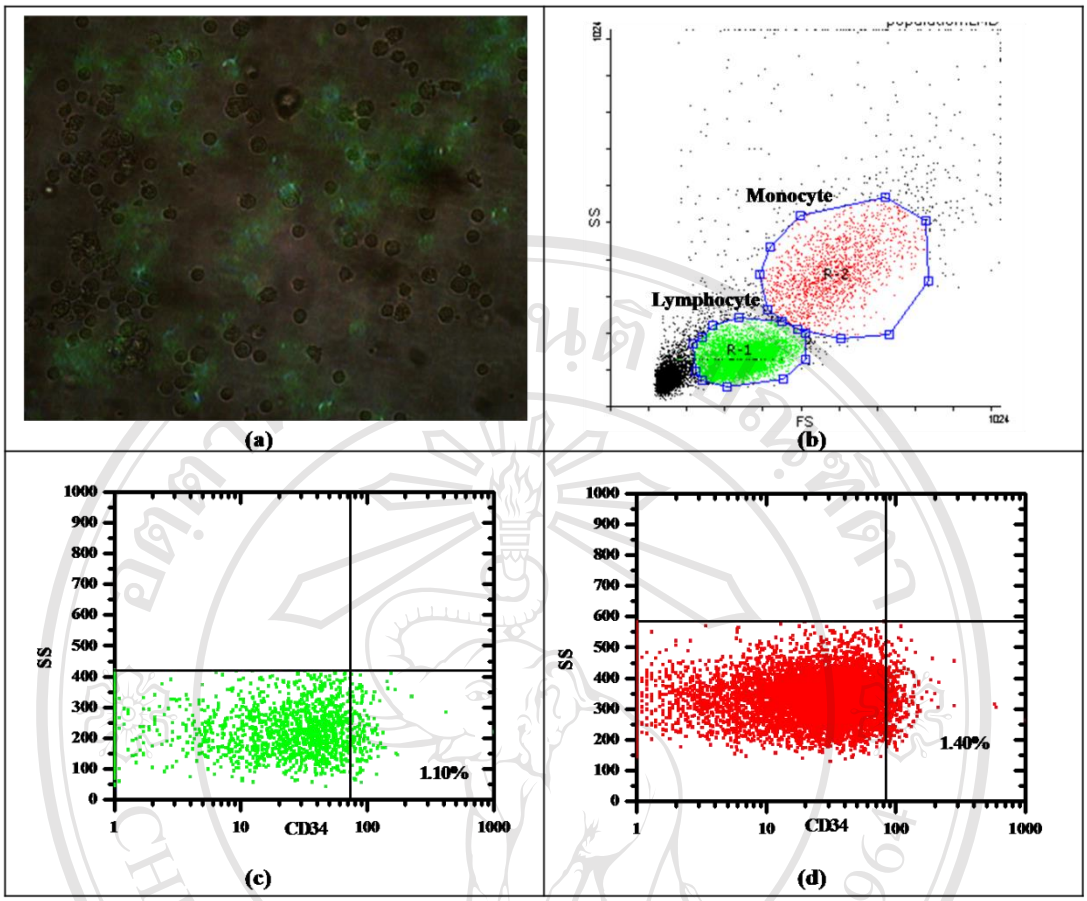

Figure 1 Characterization of peripheral blood stem cells by fluorescence micrograph (a), and cell counts using flow cytometer (b, c, d).

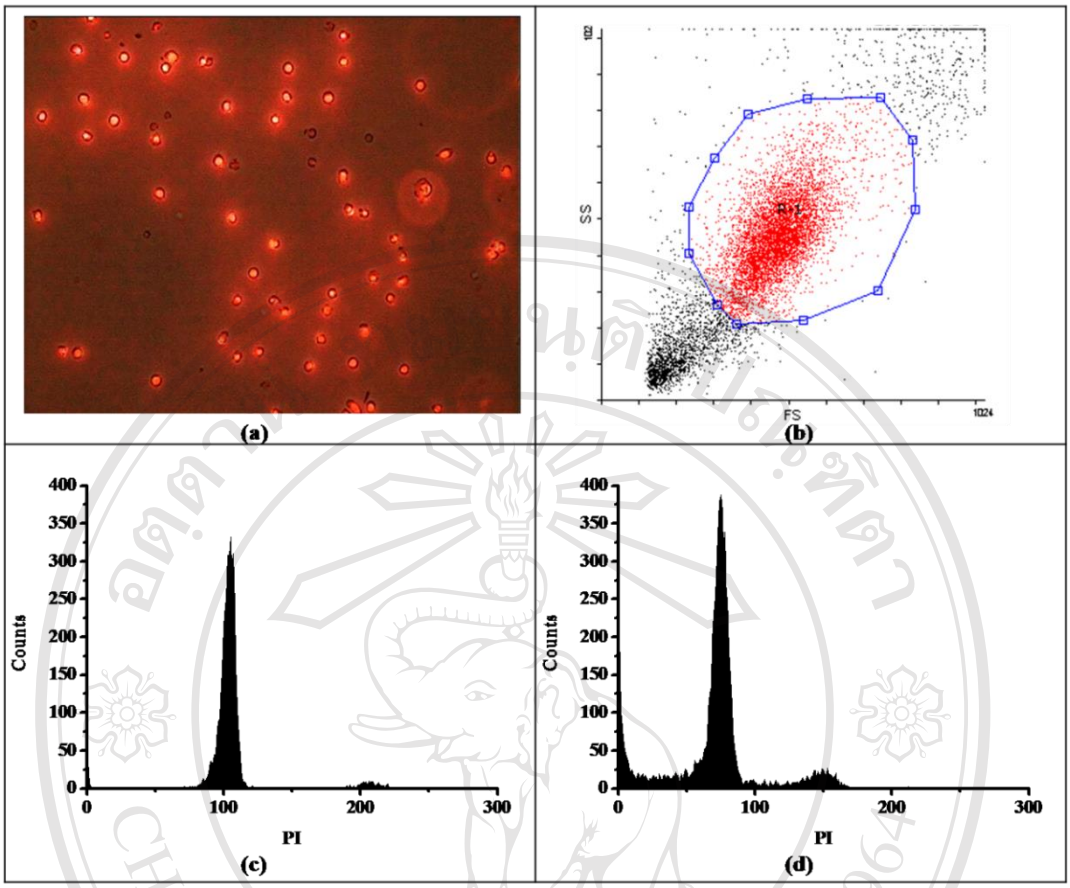

Figure 2 Characterization of peripheral blood stem cells by fluorescence micrograph (a), and cell counts using flow cytometer (b), and number of proliferating cells as a function of culture time (c, d).

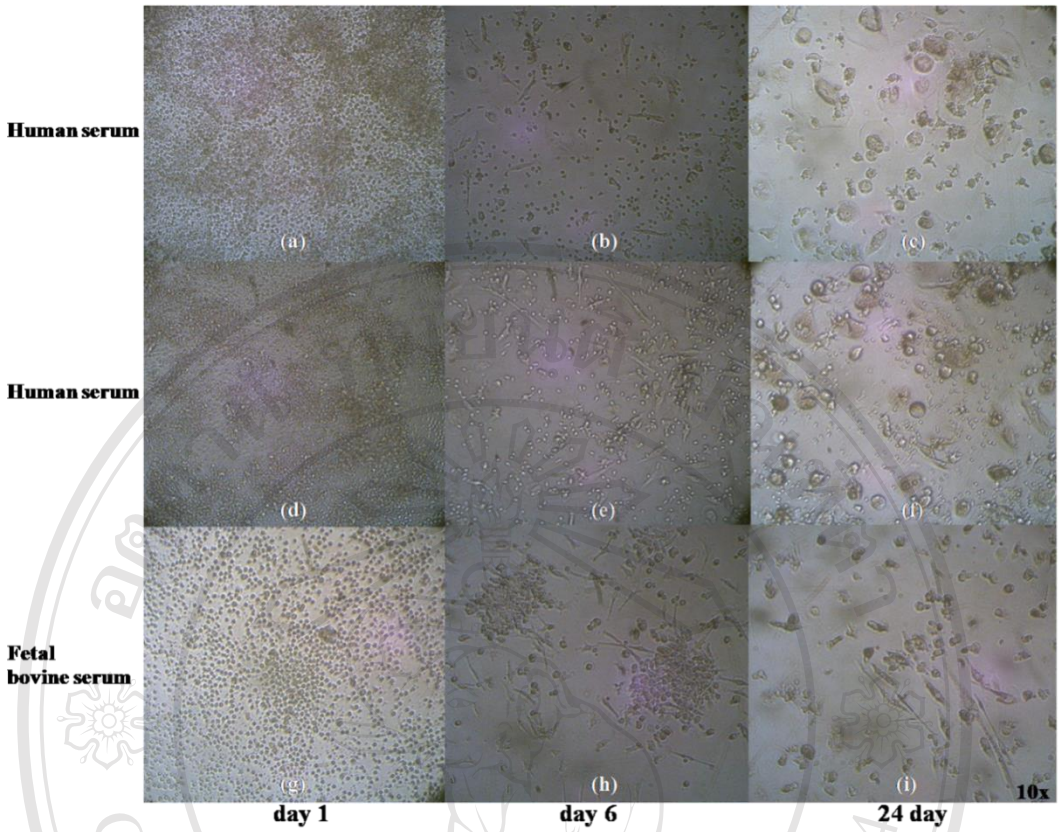

Figure 3 Micrograph of peripheral blood stem cells at day 1, day 6, day 24 after seeded. Serum of their own donor compared with the series using fetal bovine serum

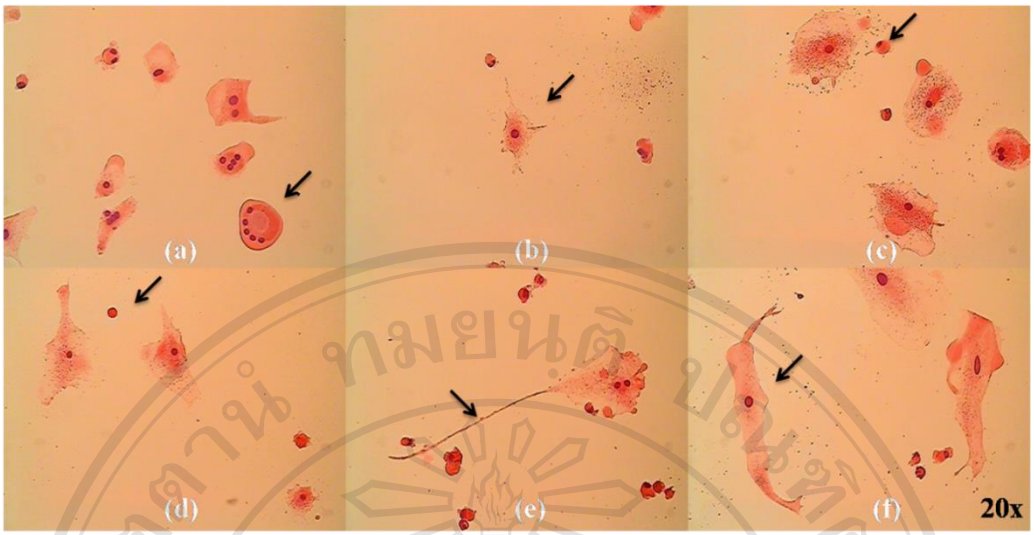

Figure 4 Micrographs of H&E staining cell in human serum; (a, f) keratinocytes, (b, c, d,) foam cells and (e) dendritic with the objective lens 20x

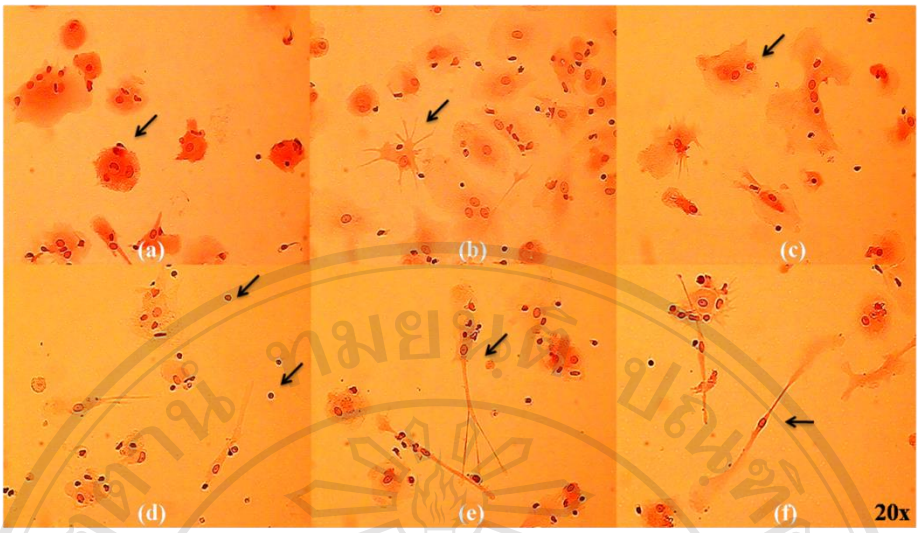

Figure 5 Micrographs of H&E staining cell in bovine serum; (a) Adipocyte, (b Osteocyte, (c) Chondrocytes, (d) Leukocyte, (e) Neuron, (f) Stromal cell at 24 day with the objective lens 20x

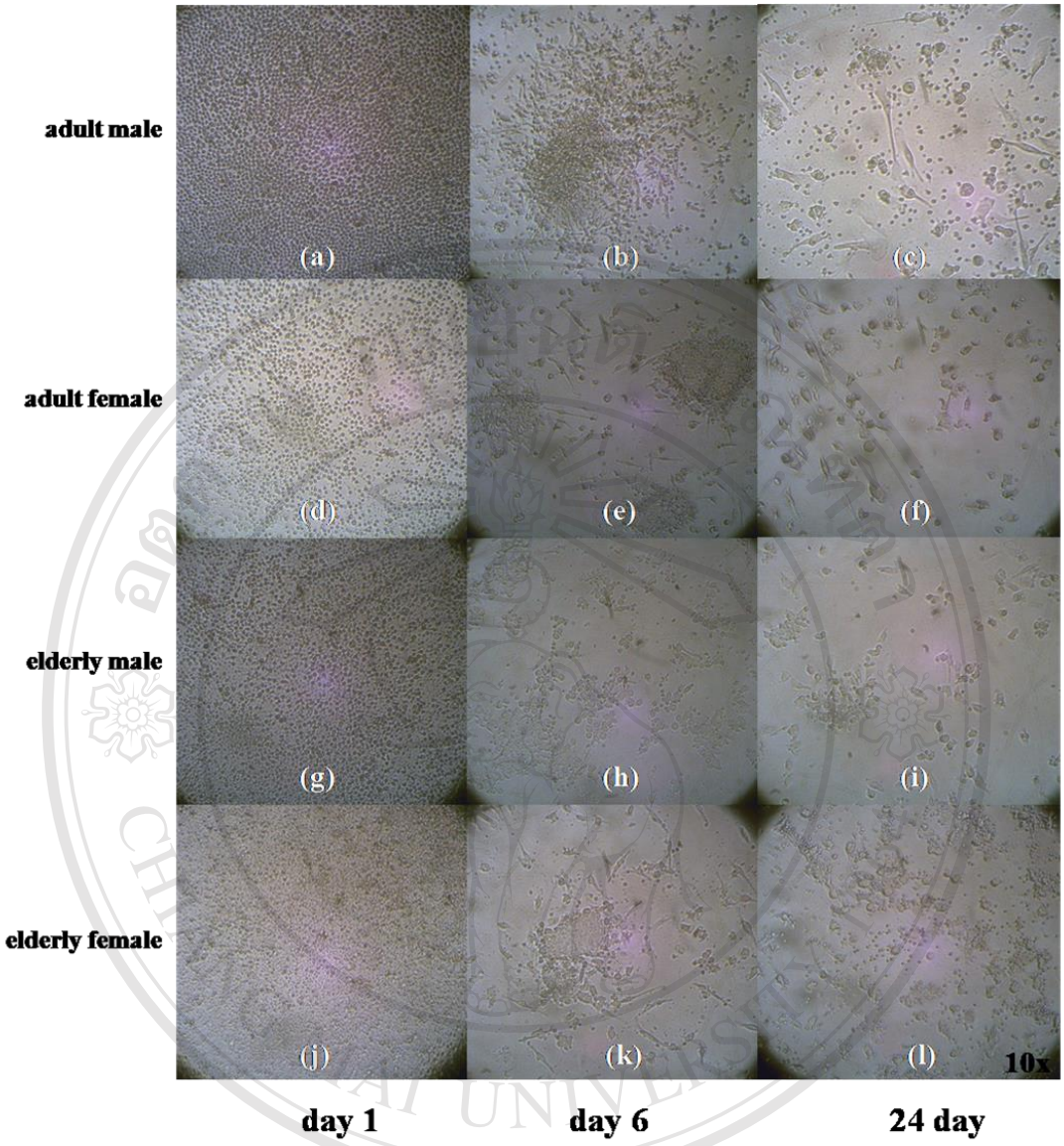

Figure 6 Micrograph of peripheral blood stem cells at day 1, day 6, day 24 after seeded. The cells were cultured using the medium completed with 10% fetal bovine serum

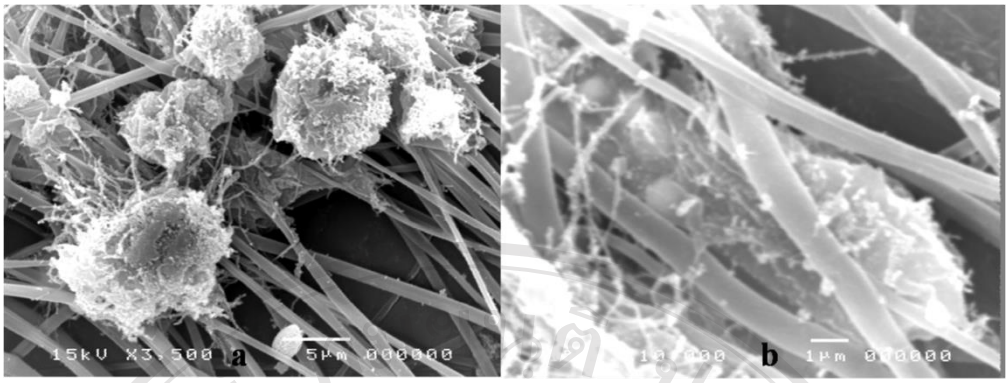

Figure 7 SEM demonstrated that the irregular forms of cell organizations (a), and the cells used the fibers as support for hanging themselves in the space (b).

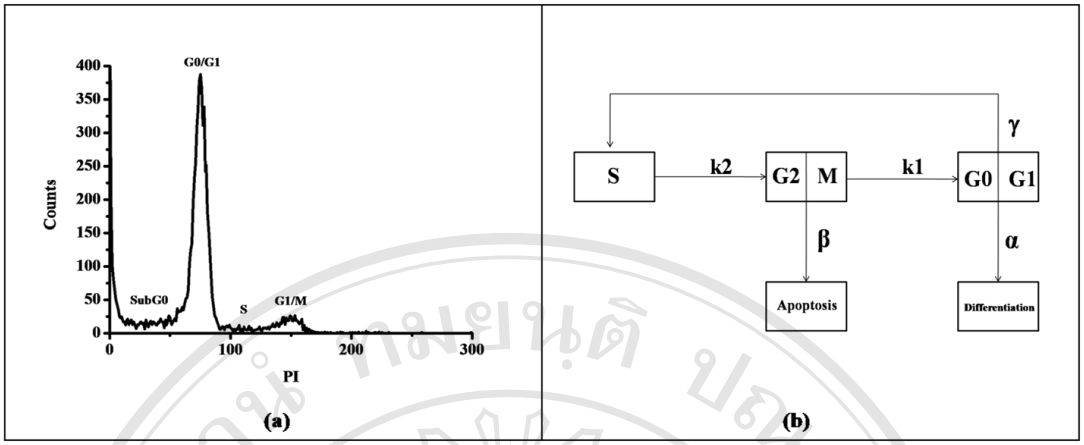

Figure 8 Number of proliferating cells as a function of culture time were found in different cell phases including G0/G1, S, G2/M and sub G0/G1 (a), Diagram explaining the homeostasis (b)

Table 1 Characteristics of PBMCs isolated from 12 donors number of PBMCs and number of S=G2/M, ($p = 0.059$ and 0.44)

Donor	Numbers of PBMCs, cell/mL	Number of S +G2/M, % of PBMCs
Adult		
AM1	**$3.7 \times 10^5 \pm 1.3 \times 10^5$**	**11.79**
AM2	**$5.0 \times 10^5 \pm 1.3 \times 10^5$**	**11.48**
AM3	**$2.4 \times 10^5 \pm 1.3 \times 10^5$**	**2.61**
AF1	**$2.5 \times 10^5 \pm 1.3 \times 10^5$**	**-**
AF2	**$2.8 \times 10^5 \pm 1.3 \times 10^5$**	**-**
AF3	**$5.4 \times 10^5 \pm 1.3 \times 10^5$**	**3.29**
Elderly		
EM1	**$4.4 \times 10^5 \pm 12.7 \times 10^5$**	**2.94**
EM2	**$3.5 \times 10^5 \pm 12.7 \times 10^5$**	**2.83**
EM2	**$8.6 \times 10^5 \pm 12.7 \times 10^5$**	**2.6**
EF1	**$6.8 \times 10^5 \pm 1.72 \times 10^5$**	**0.65**
EF2	**$4.7 \times 10^5 \pm 1.72 \times 10^5$**	**4.08**
EF3	**$3.5 \times 10^5 \pm 1.72 \times 10^5$**	**1.61**

Table 2 The determined parameters of cell kinetics including the mean rate of cell to re-entering to the cell cycle (**γ**), move from S to G2/M (k2), move from G2/M to G0/G1 (k1) undergone apoptosis (**β**) and differentiation (**α**).

Donor	**Kinetic parameter of Homeostasis**				
	k2(s)	**k1(G2/M)**	**β(SubG0)**	**α(Diff)**	**γ(G0/G1)**
AM1	2.1	4.9	0.8	1.25	4.95
AM2	24.8	8.16	0.61	24.35	8
AM3	3.32	7.7	0.45	21.82	32.4
AF1	1.1	4.2	0.8	18.9	23.4
AF2	8.8	0.002	5.4	1.702	1.7
AF3	0.175	0.084	0.2	37.541	37.6
EM1	1.2	1.04	1.518	19.678	20.4
EM2	2.36	0.35	0.72	26.81	28.8
EM3	1.72	0.51	0.18	16	18.05
EF1	2.2	6.87	0.6	0.37	8.1
EF2	0.182	0.12	2.42	3.038	0.92
EF3	3.84	0.735	3.9	15.525	16.2
Mean	**4.3±6.8 cell/day (0.17±0.28 cell/hr)**	**2.8±3.2 cell/day (0.11±0.13 cell/hr)**	**1.4±1.6 cell/day (0.05±0.06 cell/hr)**	**15.58±11.82 cell/day (0.64±0.49 cell/hr)**	**16.71±12.25cell/day (0.69±0.51 cell/hr)**

REFERENCES

[1] Asahara T, "Isolation of putative progenitor endothelial cells for angiogenesis," Science, Vol. 275 No. 5302, 1997, pp. 964-967

[2] Shang H, **"**Differentiation of human peripheral blood mononuclear cells into hepatocyte-like cells in vitro," Journal of Clinical Rehabilitative Tissue Engineering Research, Vol. 13 No. 10, 2009, pp. 1859-1863

[3] Badorff C, "Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes," Circulation, Vol. 107 No. 7, 2003, pp. 1024-1032

[4] Simper D, "Smooth muscle progenitor cells in human blood," Circulation, Vol. 106 No. 10, 2002, pp. 1199-1204

[5] Hu G, "Supernatant of bone marrow mesenchymal stromal cells induces peripheral blood mononuclear cells possessing mesenchymal features," International Journal of Biological Sciences, Vol. 7 No. 3, 2011, pp. 364-375

[6] Wan C, "Allogenic peripheral blood derived mesenchymal stem cells (MSCs) enhance bone regeneration in rabbit ulna critical-sized bone defect model," Journal of Orthopaedic Research, Vol. 24 No. 4, 2006, pp. 610-618

[7] Dellambra E, "Downregulation of 14-3-3sigma prevents clonal evolution and leads to immortalization of primary human keratinocytes," Journal of Cell Biology, Vol. 149 No. 5, 2000, pp. 1117-1130

[8] Horschitz S, "Generation of neuronal cells from human peripheral blood mononuclear cells," Neuroreport, Vol. 21 No. 3, 2010, pp. 185-190

[9] Fahey TR, "Cytokine production in a model of wound healing: the appearance of MIP-1, MIP-2, cachectin/TNF and IL-1," Cytokine, Vol. 2 No. 2, 1990, pp. 92-99

[10] J. Yu, "The predictive value of white cell or CD34+ cell count in the peripheral blood for timing apheresis and maximizing yield," Transplantation, Vol. 39 No.5, 2002, pp. 442-450

[11] Anderson DJ, "Can stem cells cross lineage boundaries?," Nature Medicine, Vol.7 No. 4, 2001, pp. 393-395

[12] Bensinger WI, "Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor," Stem Cells, Vol. 14 No. 1, 1996, pp. 90-105

[13] Zhang Z, "In vivo study on the potential differentiation capabilities of human peripheral blood-derived mononuclear cells transplanted into the nude mice with myocardial infarction," Journal of Biomedical Engineering, Vol. 25 No. 2, 2008, pp. 424-428

[14] Wolfgang Wagner, "Aging of hematopoietic stem cells is regulated by the stem cell niche," Experimental Gerontology, Vol. 43 No. 11, 2008, pp. 974–980

[15] C. Zhang et al, "Mobilization of peripheral blood stem cells for autologous transplantation patients with hematological malignancies: Influence of disease, mobilization method, age and sex," Transfusion and Apheresis Science, Vol. 39 No. 1, 2008, pp. 21–28

[16] Metallo CM, "Engineering the stem cell microenvironment," Biotechnology Progress, Vol. 23 No. 1, 2007, pp. 18–23

[17] Hkmet Geckil, "Engineering hydrogels as extracellular matrix mimics," Nanomedicine, Vol. 5 No. 3, 2010, pp. 469–484

[18] Doyonnas R, "Hematopoietic contribution to skeletal muscle regeneration by myelomonocytic precursors," Proceedings of the National Academy of Sciences of the united states of America, Vol. 101 No. 37, 2004, pp. 13507-13512

[19] Orlic D, "Mobilized bone marrow cells repair the infarcted heart, improving function and survival," Proceedings of the National Academy of Sciences of the united states of America, Vol. 98 No. 18, 2001, pp. 10344-10349

[20] Sigurjonsson O, "Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord," Proceedings of the National Academy of Sciences of the united states of America, Vol. 102 No. 14, 2005, pp. 5227-5232

[21] Susanne Grassel, "Influence of cellular microenvironment and paracrine signals on chondrogenic differentiation Reconstitution of cell communities and tissues of normal," Bioscience, Vol. 12 No. 1, 2007, pp. 4946-4956

[22] Karen Bieback, "Replicative aging and differentiation potential of human adipose tissue-derived mesenchymal stromal cells expanded in pooled human or fetal bovine serum," Cytotherapy, Vol. 14 No. 5, 2012, pp. 570-583

[23] Clifford D.L Folmes, "Metabolic Plasticity in Stem Cell Homeostasis and Differentiation," Cell Stem Cell, Vol. 11 No. 5, 2012, pp. 596-606

CURRICULUM VITAE

Author's Name Mr. Nutthapong Moonkum

Date/Year of Birth 23 December 1986

Place of Birth Mea Hong Son, Thailand

Education

2004 Certificated of High school Yupparaj Witthayalai, Chiangmai, Thailand

2008 Bachelor of Science Chiang Mai University, (Radiologic Technology) Chiang Mai, Thailand

2012 Master of Science Chiang Mai University, (Medical Radiation Sciences) Chiang Mai, Thailand