CHAPTER IV

Discussion and Conclusion

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 [37], heart [38], and neurons [39]. 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 [40]. 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 [36].

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 [41]. 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.