CHAPTER IV

DISCUSSION AND CONCLUSION

Adult peripheral blood stem cells (PBSCs) are found in normal subject's blood circulation about 0.1% (of total white blood cells). 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. Contrary to huge reports accumulated since the last decade that researchers were focused on cytokines, stromal cells and combined these two studied fields to optimize the experimental conditions for hematopoietic stem cell expansion (Choi et al., 2004; Fukushima & Ohkawa, 1995; Harvey & Dzierzak, 2004; Kadereit et al., 2002; Koh et al., 2005). The PBSCs can be concentrated in the PBMCs fraction of ficoll gradient centrifugation and were characterized by using the immunological analysis of *CD 34*$^{+}$ marker which is currently considered to reflect the best surrogate measure of hematopoietic stem and progenitor cells and also the most precise marker to guide timing of peripheral blood stem cell (PBSC) apheresis (Noronha, Lorand-Metze, & Grotto, 2006; Rowley et al., 2001). The results clearly showed by flow cytometric analysis revealed that the *CD34*$^{+}$ cells were found throughout the R1, R2 and R3 region but only those found in R1 region that have smallest and lowest granularity can preserve re-newal characteristic. The *CD34*$^{+}$ cells found in R2 and R3 which have bigger and higher granularity than those found R1 were differentiated to specific cell types. In fact, the smallest size of PBSCs preserve the self-renewal characteristic. The results signified that

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differentiation to a variety of specific cell types of PBSCs should be along with an increase in biomass of cells. The similar findings of self renewal and differentiation to a variety of specific cell types were reported in the human when PBSCs were used for autologous transplantation. Transplantation using PBSCs has several advantages including more rapid neutrophil and platelet recovery, and reduced platelet and red blood cell transfusion requirements (Rowley et al., 2001).

The DNA content analysis of cells revealed that the number of PBSCs in resting phase G0 at steady state was constant in our system. One fraction of cells in G0 undergo differentiation to a variety of specific cell types while the other fraction of cells leave from G0 entering to G1, S, G2 and M phase of cell cycle or the proliferating cells. These proliferating cells might undergo apoptotic cell death by which controlled the number of cells in the system the so-called “the cellular homeostasis”. In this study PBSCs can be expanded and the maximum of PBSCs which preserve the renewal property were about 4 times within 6 days compared with those at the initial situation. In conventional cell culture conditions, a variety of specific cells types such as endothelial cells, neuron cells, chondrocytes, myocytes, lymphocytes, etc. were observed.

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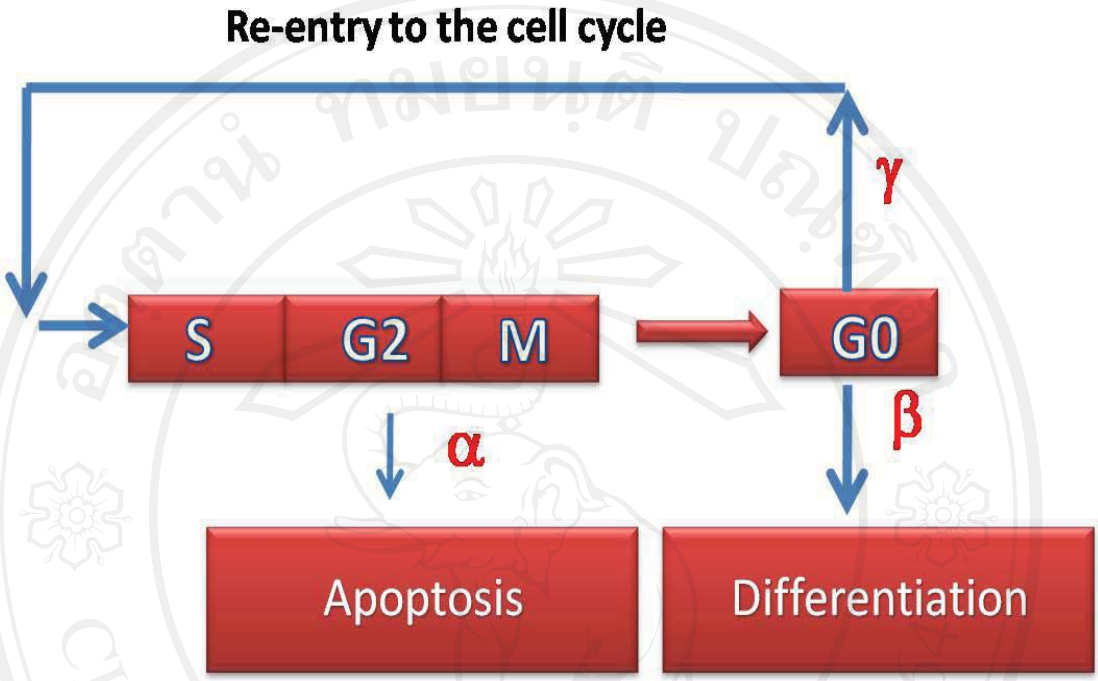

Figure 4.1 Scheme diagram of homeostasis of PBSCs in culture system

The pluripotency of PBSCs can also be demonstrated in the 3D-PVDF nanofibrous scaffold culture system. The 3D-PVDF nanofibrous scaffolds were in house fabricated by electro spun technique. The nanofibers were designed to have mixed morphology of non-woven and alignment form called the egg-net scaffold. Indeed, the mixed morphology of non-woven and alignment scaffolds has high porosity. The oval shape of egg form consists of the nanofibers lined in non-woven form generating opaque and highly porous matrices, while the net shape consist of aligned nanofibers resulting in translucent loose tissue. By using the egg-net scaffolds in the culture system, they themselves generate the specific microenvironments that influenced on the stem cell attachment, growth following cell community and tissue formations. This study show that

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the applications of the in house made of disc-like scaffolds with 250 mm diameter and 0.5 mm thickness and 3×10^6 cells in 50 μL of RPMI 1640 was the most appropriated conditions that can allow the cells to spread throughout on the scaffold surface. During cell culture, beside the cells grew on the scaffold, the suspended colonies and attached cells outside the scaffolds always present and underwent differentiation in similar way that observed in conventional culture technique. These results strongly suggested that the 3D-PVDF nanofibrous scaffolds are biocompatible and did not disturb the growth and differentiation patterns of cells. The scaffold should act as extracellular matrix supports and hangs the attached cells in the space, later develop communication and create a specific cell communities and specific tissues (Figure 3.6, 3.7, 3.8 and 3.9). These signified that the culture system used in this study should provide an appropriate microenvironments such as complex network of cytokines (stem cell factor (SCF), Flt-3 ligand (FL), thrombopoietin (TPO), leukemia-inhibiting factor (LIF), interleukin; IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, and IL-15 (Cheng, Qasba, Vanguri, & Thiede, 2000; Jang et al., 2006)), adhesion molecules, and extracellular matrix proteins that regulate survival, proliferation, growth and differentiation of PBSCs (Janowska-Wieczorek et al., 1999; Placzek et al., 2009).

The overall results suggest that the PBMCs fraction is an important of source of pluripotency stem cells which particularly have the homeostasis process for controlling the number of cells which is the characteristic of normal stem cells. These PBSCs are able to generate cell communities and tissues in the appropriate microenvironments. The results also clearly shown that the 3D- PVDF nanofibrous scaffold of both non-woven

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and alignment matrices were very good supporters that can hang the cells in space and restrictly controlled the micro environments allow the cells organizing the 3D tissues.

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