CHAPTER IV

Discussion and Conclusion

The study provides strong evidence that CESs can be found in the peripheral blood. While numbers and status of CESs dependent on health status of individuals. CESs in the body are the importance source of stem cells for body itself healing. Moreover, CESs are increasingly used as an autologous source for cell therapies, including those for the regeneration tissues including skeletal muscle[94], heart[95], and neurons[96]. The study was rigorously investigated the biological and potency of CESs obtained from 27 healthy donors and 6 donors of degenerative diseases. On one hand, the characteristics of CESs can be assessed by using conventional cell culture in combination with live cell imaging. On the microscopic analysis point of view, CESs composed of two subpopulations; one was adherent cell and the other is suspension cell. Both of cell types were able to originate the colony forming units of stem cells in the culture system but it was difficult to enumeration of the 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 3.5a 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 homeostasis diagram 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, 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 3.1. The CESs' potency in terms of cell growth and differentiation was not depending on age, gender and blood group of donors. However, their potency depending on chemical environments.

On the other hand, their potency to differentiation, repair and regeneration of bio-tissues can be assessed by culture the CESs in the presence of mixed morphology of 3D-nanofibrous PVDF scaffold. The CESs of all donors able to grow and undergo

differentiation yielding complex tissues such as bone, tubular like structure, muscle bundles, and neurovascular bundles as revealed by SEM micrographs after 10 days (figure 3.7,3.8 and 3.9). Almost the attached cells on the alignment zone were differentiated in a variety of cellular morphologies. The cells found in the non-woven zone were irregular in shape and sites. Indeed, the 3D-nanofibrous scaffold should be applied as supporter that can hang the CESs in space that allow their growth in three dimension yielding complex tissues. These bio-tissues are useful as sources of whole cells proteomics and secreted protein profiling in mimic physiological conditions. By using 2D-gel electrophoresis and LC-MS of interested proteins as well as the Panther data base, we proposed the panel proteins at least 42 proteins as biomarkers for evaluating repair and regeneration process *in vivo* situation. Among 42 proteins, there are 5 common proteins that can be found in both control group and degenerative disease group. Siamois polyphenols clearly regulated by increasing CESs and induce increases repair and regenerative biomarkers in degenerative disease group.The study also suggested that 2D-gel electrophoresis is an appropriate tool for detect biomarker in the body. The 5 common proteins including 5.Q8N6Y2 (Leucine-rich repeat-containing protein), 16. Q9UBD9 (cardiotrophin-like cytokine factor 1), 30.Q60687 (sushi repeat-containing protein precursor), 37.P04179 (superoxide dismutase [Mn]) and 38.Q8IW75 (serpin A12) can be proposed as biomarkers for evaluating efficiency of repair and regeneration process in the body level.

The alteration of the five biomarkers was studied by realizing series of experiments for plasma protein analysis of degenerative disease groups including head injury, retinal detachment, stroke and CA colon. The degenerative diseases in this study was managed by applying Siamois polyphenols (500 mg/capsule) of 2 capsules of Siamois I and 2 capsules of Siamois II for 4 times a day (1 month). The plasma protein profiling was constructed in parallel with the CESs culture. The PBMCs isolated from neurodegenerative disease group presented the residual bodies, granules, droplets and vacuole. The particular observation was for retinal detachment, the cell changed their morphology from round to irregular shape consist of fibrous like. The common types of cells obtained from degenerative group can be classified as stress-cell, transformed cell and undergo death cells. It should be noted that there were not colonies of stem cells found in the culture system.The results clearly show that the 5 proteins including protein number 5 Q8N6Y2 (Leucine-rich repeat-containing protein), 16 Q9UBD9

(cardiotrophin-like cytokine factor 1), 30 Q60687 (sushi repeat-containing protein precursor), 37 P04179 (superoxide dismutase [Mn]) and 38 Q8IW75 (serpin A12) were found as 100% common protein as healthy donors. As can be seen, the 5 proteins were down regulated in case of head injury and retinal detachment, while up regulated in case of stoke and mixed of stroke, hypertension and CA-colon. The results indicated that the 5 biomarkers are significantly revealed the health status of degenerative diseases. They can consider like down regulation in head injury and retinal detachment and up regulation in stroke and stroke include hypertension and CA colon.

Since Siamois polyphenols efficiently induce neurovascular bundle and eliminated the stress conditions in degenerative disease group that clearly demonstrated by the repair and regeneration biomarkers. Thus, the behavior of these repair and regeneration biomarkers was used for evaluating the treatment outcome of PD and MS using Siamois polyphenols. Siamois polyphenols (500 mg/capsule) of 2 capsules of Siamois I and 2 capsules of Siamois II for 4 times a day. The blood collection was performed at different time interval for CESs and plasma protein analysis. Initially, PBMCs isolated from neurodegenerative disease group presented the residual bodies, granules, droplets and vacuole. The common types of cells obtained from PD and MS volunteers can be classified as stress-cell, transformed cell and undergo death cells. It should be noted that there were not colonies of CESs found in the culture system. After 3 weeks of treatment using Siamois polyphenols, we observed the colonies of CESs and the stress and transformed cells were disappeared. After 11 and 22 week the colonies of CESs become bigger and the neurovascular bundles were observed. These results are get along with the pre-post clinical symptom score and MRI images. The results suggest that Siamois polyphenols can induce and regulate the repair and regeneration of tissue in particularly neurovascular bundles that are the basic structure of tissue formation and finally can improve motor symptom in PD and MS.

The overall results of the study showed the CESs can differentiate to varieties of specific cells in the same condition. In addition, the CESs efficiently regenerated new communities of cells and tissue in 3D-nanofibrous scaffold system. And findings demonstrated that effect of Siamois polyphenol exert neuroprotective effect through enhancement of CESs and improve the clinical symptom. Thus Siamois polyphenols should considered as promising compound for treatment of the neurodegenerative disease and might be potential used for the treatment of another degenerative diseases.