CHAPTER I

Introduction

Neurodegeneration is the progressive loss of structure or function of neurons including death of neurons with limitation of functional and physiological ability. This impairment of neuromuscular function leading to a progressive decrease in everyday activities and a reduction in quality of life. Recent knowledge indicates the possibility that such impairment of neurological diseases caused by perturbance and/or damages of neurons can be regenerated by stem cells.[1]However, the mechanism and explanation of the regeneration processes remain to be elucidated. Many research groups aim to explore the role of endogenous stem cells in tissue and organ repairing such as wound healing,[2] spinal cord,[3]heart tissue[4] and brain tissue injuries.[5] In our research group, we are working on the identification and characterization of circulating endogenous stem cells by monitoring stem cells behavior under the assessment of optical imaging with immunostaining technique. The results indicated that the stem cells isolated from the peripheral blood can transform into several kinds of cells that look alike cardiacmuscle,[6] bone,[7] neuronal system[8] and vascular system.[9] The potency of the stem cells to generate tissue depend up on physicochemical properties of the microenvironment. We have found that the stem cells in blood circulation compose of hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). These stem cells were found in different states of development and the precursor cells are proposed to migrate to specific area by which guiding by cytokines derived from neighbor cells of damaged lesions. These cytokines are such as Granulocyte-colony stimulating factor (G-CSF), Granulocyte macrophage colony stimulating factor (GM-CSF) and stromal derive growth factor (SDF).[10]In fact, the microenvironment of our body plays a crucial role on stem cell growth and differentiation. It should be noted that the stem cells isolated from blood

circulation the so-called circulating endogenous stem cells are very sensitive to oxidative stress conditions.[11]As can clearly be demonstrated that the circulating endogenous stem cells proliferation and their potency was decreased in the oxidative stress conditions (see result section). This is the limiting step leading to a decrease in the number of circulating endogenous stem cells found in the blood circulation of adult people. In order to convey stem cells to the lesion of interests, it is of importance to increase numbers of circulating endogenous stem cells in blood circulation and improve the chemical environment of blood appropriate for circulating endogenous stem cells retention and differentiation to precursor cells. We thus proposed strategies to resolve these limitations as followed. The first is an alteration of the immune system and the second is an elimination of the origins of oxidative stress that contributes by stress cells, transform cells and pathological cells.

1.1 Circulating endogenous stem cells (CESs)

Circulating endogenous stem cell (CESs) is a large accessible source of adult stem cells. These endogenous stem cells are clearly found in all adult tissues. They reside in specific microenvironment of tissue the so-called "stem cell niche" and triggered by neighbor cells of damaged tissue then leaving from the niche to blood circulation. Consequently, these stem cells will be recruited and brought to lesions and participating to repair and regeneration processes. In human body, CESs are regarded as readily available reservoirs of cells that able to mobilize, proliferate and differentiate to the appropriate cell type in response to specific signals then undergo repair and regeneration[12]. Indeed, they are the sources of stem cells for body heals itself such as wound healing, myocardial regeneration, angiogenesis, neurogenesis and immunity system[13].However, the repair and regeneration processes are crucial dependent on the microenvironments of the tissue. Unfortunately, these variable parameters that influencing the efficiency of CESs repair and regeneration still need to be elucidated. Many studies reported that possibilities to expand the stem cells in culture systems and tried to find out the ways that can promote stem cells homing[14] in particularly, the research on cytokines that induce differentiation of stem cells to precursor cells. The affecting parameters such as aging[15], gender[16]and microenvironment of cell culture[17] were also proposed to be the key factors of the potency of the stem cells especially

effect on their behavior and morphology. Indeed, human body is a very complex system and cannot be treated one or some parameters as trying to deal with in those studies. It is a need to do *ex-vivo* investigation of the potency and repair and regeneration role of endogenous stem cells by respecting to their health status at different time intervals. After expansion by using cell culture these endogenous stem cells are multitude of distinct multipotent cells that are able to differentiate in almost cell types during culture in specific condition medium including blood cells, endothelial cells[18], hepatocytes[19], cardiomyogenic cells[20], muscle cells[21], osteoclasts[22], osteoblasts[23], epithelial cells[24], neural cells[25]and myofibroblasts[26]. However, it was very difficult to compare the efficiency and capability of the stem cells of different sources reported in international literature due to the different methods of isolation and expansion used of each research group.

1.2 Degenerative disease

A degenerative disease is a type of a medical condition that causes a tissue or organ to deteriorate over time. There are quite a number of degenerative diseases and many of them are associated with aging, or gets worse during the aging process[49].Degenerative diseases are classified into three main groups: cardiovascular, neoplastic, and nervous system. The most common cardiovascular diseases are hypertension, coronary disease, and myocardial infarction. Neoplastic diseases include tumours and cancer. Diseases that affect the nervous system include Parkinson's and Alzheimer's.[32]Degenerative diseases are caused by a wide variety of factors. Some are a direct result of normal wear and tear of the body, while others are perpetuated by poor health or an unhealthy lifestyle.

Many degenerative diseases can be cured, but there are still quite a few that have no treatment. In such cases, the available options are designed to relieve the symptoms to help patients have a normal life as much as possible.They discovered that a key similarity is the presence of abnormal proteins. However, precisely how these abnormal proteins cause cell deterioration is still a mystery. Solving this mystery will pave the way to improve the current available treatment options or create new treatments that not only relieve the symptoms, but also cure the disease itself.

1.3 Neurodegeneration

Neurodegenerative diseases are characterized by progressive dysfunction of specific population of neurons. The neurodegenerative diseases includes Huntington's disease (HD),[27] Multiple sclerosis (MS),[28] Parkinson's disease (PD),[29] Alzheimer's disease (AD)[30] and Peripheral neuropathy (PN).[31] Major basic processes these neurodegenerative disease are abnormal protein dynamics due to deficiency of the ubiquitin-proteosome-autophagy system, oxidative stress and free radical formation, mitochondrial dysfunction, impaired bioenergetics, dysfunction of neurotrophins, neuroinflammatory processes and disruptions of neuronal Golgi apparatus and axonal transport.[32]All neurodegenerative disease have similarities of pathological at a sub-cellular level. These interrelated mechanisms lead to programmed cell death is a long run over many years.[33] Neurodegeneration can be found in different levels of neuronal circuitry ranging from molecular to systemic.The discovery of these common pathological offers hope for therapeutic advances to ameliorate the neurodegenerative diseases.

In this work were interested in the potential of CESs regeneration of neuronal tissue in high degree of damaged central nervous system (CNS) that now cannot cure by current regimen of treatment such as tremor-dominant Parkinson's disease (PD) and Multiple sclerosis (MS)**.**

1.3.1 Parkinson's disease

Parkinson's disease is the one of neurodegenerative disease that initially described by James Parkinson's initial description in the 19th century, later refined by Jean-Martin Charcot.[34] The classical parkinson's disease was identified as early prominent death of dopaminorgic neurons in the substantia nigra pars compacta (SNpc) which clearly demonstrated by Axial T2 MR images of midbrain (figure 1). The resultant dopamine deficiency within the basal ganglia leads to a movement disorder characterised by classical parkinsonian motor symptoms.[35]These parkinsonian symptoms include bradykinesia, muscular rigidity, rest tremor, and postural and gait impairment.[36]Up to date, the causes of Parkinson's disease remain

unknown, but risk of developing Parkinson's disease seems to result from a complicated interplay of genetic and environmental factors affecting numerous fundamental cellular processes. The overall symptoms of Parkinson's diseases were reviewed as indicated in figure 2.

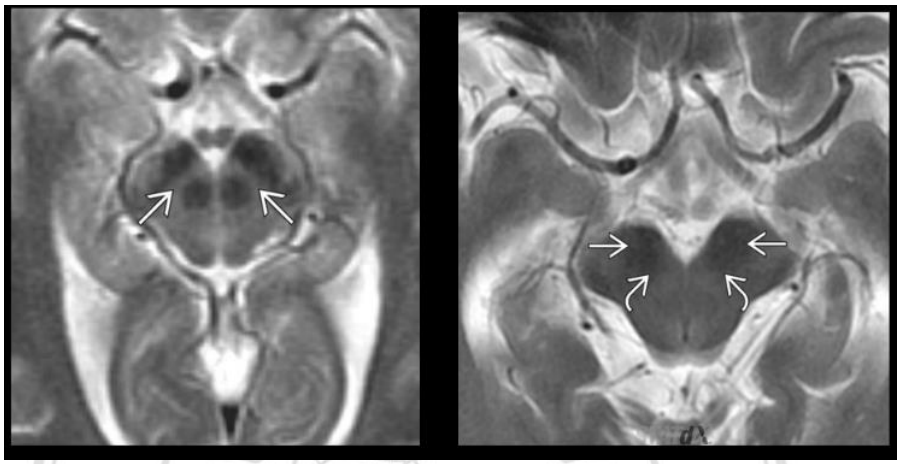

Figure 1 Axial T2 MR images compare normal midbrain with Parkinson's disease midbrain. The second image shows blurring and thinning of pars compacta. Subsequently, the red nuclei and substantia nigra are almost touching.[34]

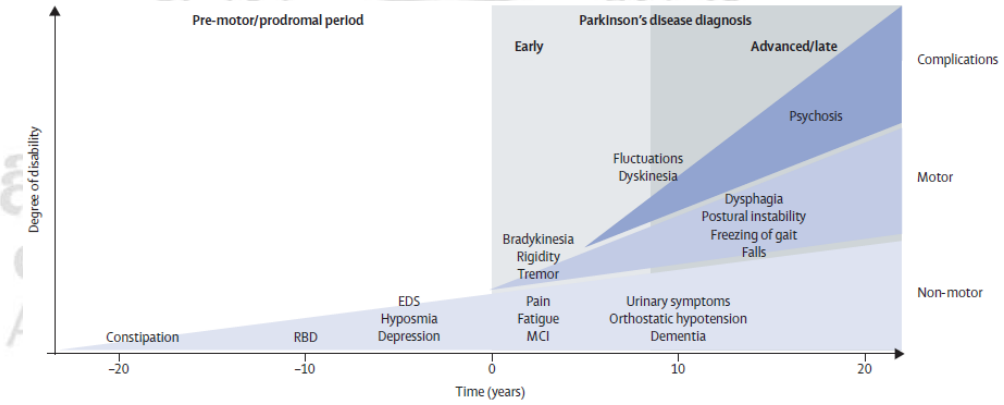

Figure 2 Clinical symptoms and time course of Parkinson's disease progression. Diagnosis of Parkinson's disease occurs with the onset of motor symptoms (time 0 years) but can be preceded by a premotor or prodromal phase of 20 years or more. This prodromal phase is characterised by specific non-motor symptoms. Additional non-motor features develop following diagnosis and with disease progression, causing clinically

significant disability. Axial motor symptoms, such as postural instability with frequent falls and freezing of gait, tend to occur in advanced disease. Long-term complications of dopaminergic therapy, including fluctuations, dyskinesia, and psychosis, also contribute to disability. EDS=excessive daytime sleepiness. MCI=mild cognitive impairment. RBD=REM sleep behavior disorder.[35]

A consensus on the classification of Parkinson's disease subtypes has not yet been established, but empirical clinical observations suggest two major subtypes: tremor-dominant Parkinson's disease (with a relative absence of other motor symptoms) and non-tremor-dominant Parkinson's disease (which includes phenotypes described as a kinetic-rigid syndrome and postural instability gait disorder). An additional subgroup of patients with Parkinson's disease has a mixed or indeterminate phenotype with several motor symptoms of comparable severity. Course and prognosis of disease differ between the subtypes; tremor-dominant Parkinson's disease is often associated with a slower rate of progression and less functional disability than non-tremor-dominant Parkinson's disease.[37] Furthermore, the various Parkinson's disease subtypes are hypothesized to have distinct etiologies and pathogenesis.[38] Non-motor features include olfactory dysfunction, cognitive impairment, psychiatric symptoms, sleep disorders, autonomic dysfunction, pain, and fatigue. These symptoms are common in early Parkinson's disease[39] and are associated with reduced health-related quality of life.[40,41]

1.3.2 Multiple sclerosis

Multiple sclerosis (MS) is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged.[42] This damage disrupts the ability of parts of the nervous system to communicate, resulting in a range of signs and symptoms, including physical, mental and sometimes psychiatric problems.[43] Specific symptoms can include double vision, blindness in one eye, muscle weakness, trouble with sensation, or trouble with coordination.[43] MS symptoms vary depending on the area of

the CNS affected. Based on symptoms onset and their evolution, MS phenotypes were initially described: relapsing–remitting MS (RRMS), secondary-progressive MS, primary-progressive MS, and relapsing-progressive MS (figure 3).[44] MS is usually diagnosed based on the presenting signs and symptoms and the results of supporting medical tests.[42]While the cause is not clear, the underlying mechanism is thought to be either destruction by the immune system or failure of the myelin-producing cells. Proposed causes for this include genetics and environmental factors such as being triggered by a viral infection.[42]

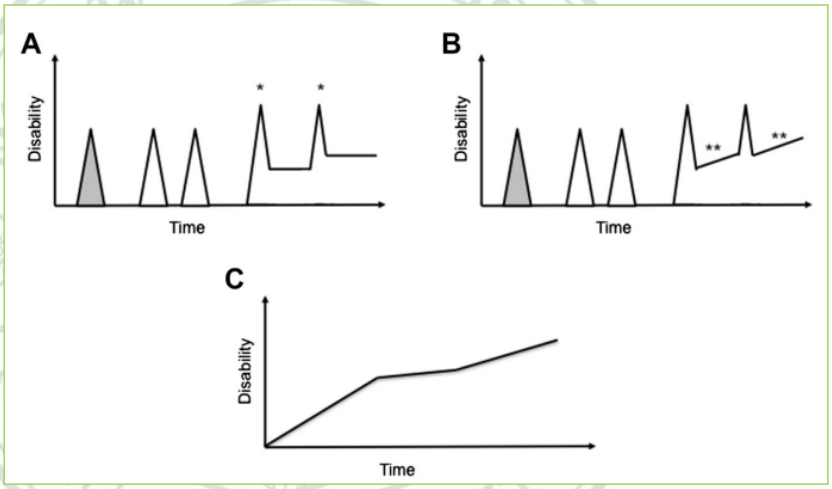

Figure 3 Multiple sclerosis (MS) phenotypes. The figure shows the different MS phenotypes. In relapsing-remitting MS (A), after the first event or clinically isolated syndrome (light gray) new relapses will occur. The recovery of these relapses may be complete or partial (asterisk). After this initial period, some patients enter a progressive phase of the disease (double asterisks), with or without superimposed relapses that constitutes the secondary progressive MS (B). In primary progressive MS (C), patients present a sustained and progressive neurologic impairment since onset.[29]
Magnetic resonance imaging (MRI) of the brain and whole spine are useful in the diagnosis and treatment outcome assessment of multiple sclerosis (MS) (figure 4)[45]

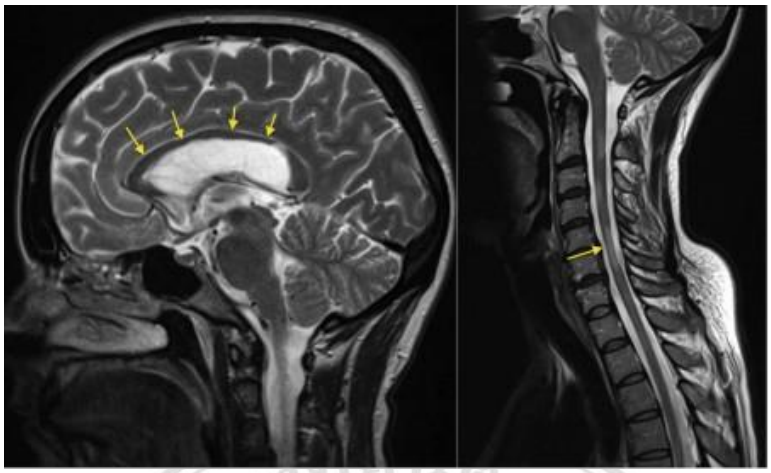

Figure 4 Multiple sclerosis-spinal cord atrophy. One of the imaging features of advanced multiple sclerosis is atrophy of the corpus callosum, illustrated in the sagittal T2-weighted image on the left here (arrows). Note the bright CSF. The patient also had multiple high-signal plaques in the cervical spinal cord, seen on the sagittal T2-weighted cervical spine image on the right. Note the focal area of thinning of the spinal cord (arrow), due to atrophy.[46]

1.4 Regeneration in neurodegenerative disorders

Most of neurodegenerative disorders were finally associated to both deleterious microenvironments resulting in neuronal and supporting cell death. Such deleterious microenvironments were triggering by various stimuli such as cytokines, chemicals, physicals, and pathogens. Consequent progressive generating oxidative stress conditions the principal causes of chronic inflammation, cell death and tissue damage. Many reports suggested that such kind of deleterious situation similar to neurodegenerative disorders might be automatically resolved such as wound healing,[2] spinal cord injury,[3]heart tissue[4] and brain tissue.[5]It is possible that in case of neurodegenerative disorders should be resolved by applying the common knowledge of neurogenesis. This should begin from endogenous neuroprotection leading to neuroplasticity and neurorestoration. Regeneration in the central nervous system (CNS) implies that new neurons, generated either through the proliferation of endogenous stem/progenitor cells or by administration of exogenous stem/precursor cells with potential to substitute for lost tissue, will differentiate, survive, and integrate into existing neural networks.[47]

In fact, there is lacking of methodologies for investigation of neurogenesis *in vivo* situation. Recent research discovered the growth factors and their pro-survival effect led to a closer investigation of specific nervous system cytokines. The growth factors are Nerve Growth Factor (NGF), Brain-Derived Nerve Factor (BDNF), and Glial-Derived Nerve Factor (GDNF). Interestingly, different neuronal subpopulations require different growth factors to thrive, for example NGF protects cholinergic neurons from various insults,[48] whereas for dopaminergic neurons, this effect is better sustained by BDNF.[49]Neuroplasticity is substrate for learning and memory formation, cognitive abilities progressively lost in AD and in late stages of PD.[50]According to Thickbroom and Mastaglia, the molecular mechanisms underlying neuroplasticity are both neuronal and nonneuronal and, furthermore, neuronal plasticity may be synaptic or non-synaptic.[51]

At the base of initial neurorestoration attempts lies the idea of enhancing the endogenous neuroprotective effect of growth factors in the CNS. At first, genetically modified fibroblasts to produce either BDNF, or NGF have been transplanted in laboratory rats and primates. The experiments were successful in rescuing functional and cellular loss. The same type of experiment was conducted, in 2005, on human patients, diagnosed with AD.[50] The delivery system consisted of induced pluripotent stem cells (iPS), generated from the recipient's fibroblast population and genetically modified into secreting NGF.

1.5 Polyphenols and neurodegenerative diseases

Natural polyphenols are the most commonly found chemical compounds in consumed herbal beverage and food worldwide.[51] They constitute a large group of photochemical with more than 8000 identified compounds.The primary function of these compounds is protection of plants against reactive oxygen species (ROS), produced during photosynthesis, and consumption by herbivores.[52] Within the previous decades, most of the studies on polyphenols have been focused on anti-oxidant properties of these chemical compounds as their most prominent effect.[53] Along with introducing resveratrol, as a potential anti-aging agent, much focus has been placed on the protective effects of various polyphenols against aging and related neurodegenerative diseases.[54] Increase in life span by polyphenols can be associated with increased or

improved brain function. For instance, epigallocatechin gallate (EGCG) postponed the onset of neurological symptoms and prolonged life span in a mice model of amyotrophic lateral sclerosis (ALS).[55] Long term treatment with epigallocatechin gallate increased the life span and enhanced movement abilities in a transgenic Drosophila melanogaster model of PD.[56] Despite the prominent evidence of neuroprotective effects of polyphenols from *in vitro* and preclinical models, overall success in bringing these compounds into routine clinical application has been limited**.**[57]

One of the most important aspects of current polyphenol research is the focus on the neuroprotective capacity that is a characteristic feature of this broad family of compounds. There is increased interest in uncovering efficient antioxidants to reduce the risk of AD, PD, and other neurodegenerative disorders, since current therapeutic approaches are merely symptomatic, without any disease-modifying activity. Because many diseases of ageing can be directly linked to repeated oxidative stress and chronic inflammation, therapies that can diminish such effects have become an important tool in seeking more effective treatments for diseases such as Alzheimer's disease, Parkinson's disease and other neurodegenerative disorders.[58]

1.5.1 Siamois polyphenols as potent apoptosis inducer, immunomudulatorand endogenous stem cells regulator

The molecular and cellular pharmacology of Siamois polyphenols attempting to apply in cancer treatment research was conducted in our research groups since 1997. Indeed, the Siamois polyphenols are very good apoptotic inducers, strong immunomodulators and strong anti-oxidant activity.[59,60] The Siamois polyphenols are widely efficiently induce both transformed and cancer cells to undergo apoptosis.[61,62]For example, 5□M (equivalent to quercetin) can induce cancer cells by 50% apoptosis cells such as erythromyelogenous leukemic and lung cancer cells.[59] Recently, Siamois polyphenols were suggested to be used as therapeutic agents for cancer treatment.[64,65]

It was also found that Siamois polyphenols play crucial role on protection of PBMCs from free-radicals.[59] *In vivo* studies, the Siamois polyphenols can

increase the number of circulating endogenous stem cells in vitro after 1 week of treatment and Siamois polyphenols play important role on the regulation of physiochemical properties of blood resulting to an appropriate situation for stem cells circulation and growth.[59,60] The results lead us to hypothesize that Siamois polyphenols should be useful for preparing individuals before starting the regeneration program of degenerative disease treatment.

1.5.2 Siamois polyphenols and circulating endogenous stem cells

Endogenous stem cells are often localized in a specific niche that is essential to maintain their property of self-renewal and differentiation.[66] A small fraction of stem cells are found in blood circulation. Ultimately, their normal function could be to maintain a constant number of cells in an organ. However, the clinical use of endogenous stem cells is currently limited for diverse reasons. Their existence remains controversial in several organs, especially in humans where lineage tracing or reconstitution experiments are impossible. Therefore, their identification and characterization is difficult and past experience has shown that results obtained in animal models might not be translated directly to humans. This limits the study of the mechanisms controlling the self-renewal and differentiation of endogenous stem cells, which is necessary for the development of future clinical treatments.

Recently, our research group have developed a 3D-nanofibrous scaffold model that mimic to physiological environment, allowed investigating the behavior of stem cells and their potency of self-renew and originating new tissues. Preliminary studies found that after orally administration 500 mg Siamois polyphenols, four hours interval, four times daily, for 15 days can efficiently eliminate the transformed and cancer cells in volunteers of cancer patient group. The results also indicated that Siamois polyphenols were efficiently depleted the stress oxidative and inflammation level in plasma. While the analysis of endogenous stem cells in blood were found to increase in numbers and types. These stem cells were high potency can differentiate

into all the cell types constituting various tissues in our *ex vivo* cell culture that mimic to physiological conditions. We also investigated biomarkers of cellular process indicating neurogenesis. By using 3D-nanofibrous scaffold in combination with video-optical imaging technique and the analysis of biomarkers of cellular process of neurogenesis can be used for investigating the role of endogenous stem on neurorestauration and neuroplasticity *in vivo* situation.

The endogenous stem cells found in the blood circulation of each individual are available homing to the specific lesion guiding by cytokines such as Granulocyte-colony stimulating factor (G-CSF), Granulocyte macrophage colony stimulating factor (GM-CSF) and stromal derive growth factor (SDF).[67] As mentioned earlier the endogenous stem cells composes of HSCs and MSCs and both of them should work as co-between in signaling and repairing of the damaged tissues. Especially, we found that the microenvironment of our body plays a crucial role on the stem cells growth and differentiation. As can be seen in figure 5, the HSCs found in blood should be in mixed population and different stages of development. Common lymphoid stem cells give rise to a class of leukocytes known as lymphocytes, which include the various T cells, B cells, and natural killer (NK) cells. Briefly, lymphoid stem cells quickly migrate from the bone marrow to lymphatic tissues, including the lymph nodes, spleen, and thymus, where their production and differentiation continues. B cells mature in the bone marrow, while T cells mature in the thymus and common myeloid stem cells give rise to all the other formed elements, including the erythrocytes; megakaryocytes that produce platelets; and a myeloblast lineage that gives rise to monocytes and three forms of granular leukocytes: neutrophils, eosinophils, and basophils. There were studies reported that the stem cell of adult subject is ranging from 0.1-1% of leukocyte.[68]In fact the numbers of stem cells in blood were depending on the physicochemical properties of blood.[69] As recently demonstrated by our research group the stem cells of an individual treating with Siamois polyphenols, vary from 3-50% of mononuclear cells. It was clear that the

stem cells derived from the subject can efficiency, give colonies and generate the new tissues in culture laboratory.[59,60] In figure 6 mesenchymal stem cells (MSCs) or marrow stromal cells are adult stem cells with multi-lineage potential that are easily genetically modified and demonstrate immunosuppressive ability. Their versatility holds promise for tissue engineering, regenerative medicine and immunotherapy. MSCs can be isolated from various tissues, including bone marrow, adipose tissue, and blood including peripheral and umbilical cord blood. They are capable of differentiating into bone, cartilage, muscle, marrow, tendon, adipose, and connective tissues.

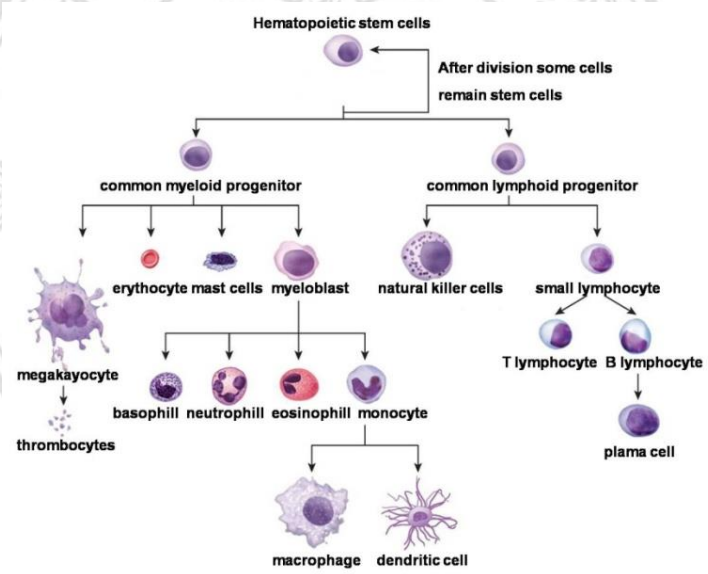

Figure 5 The process of hematopoietic involves the differentiation of multipotent cells into blood and immune cells. The multipotent hematopoietic stem cells give rise to many different cell types, including the cells of the immune system and red blood cells.[70]

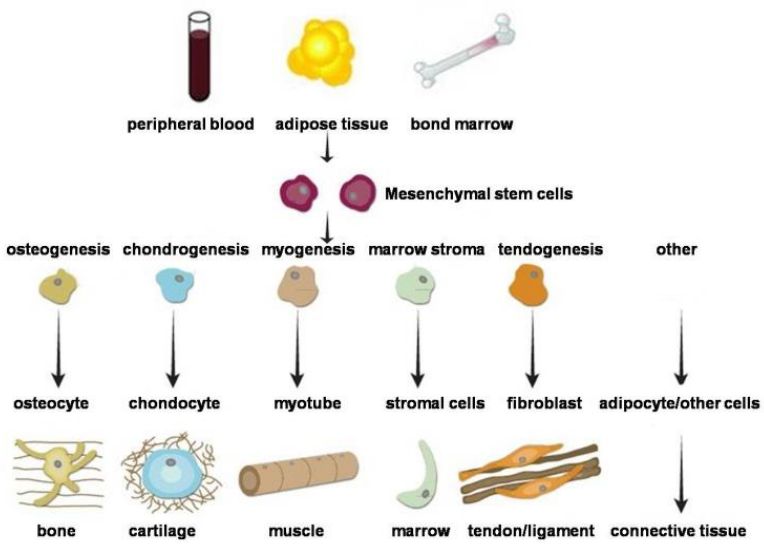

Figure 6 Mesenchymal stem cells undergo mesengenic process in order to transform into different cell types such as osteocytes (bone cells), chondrocytes (cartilage cells), muscle cells, and others.[71]

1.6 Identification and characterization of circulating endogenous stem cells

Identification and characterization of stem cells can be a challenging and often evolving process. Stem cells not only must display the appropriate markers, but a healthy and robust stem cell population must also lack specific markers as well. To add to the complexity of this area of stem cell biology marker profiles change based on the species, site of origin, and maturity of the desired population. In addition, stem cell populations may be consisted of several specific phenotypes which are often indicators of the population's general health.

Peripheral blood is a large accessible source of adult stem cells for both basic research and clinical applications. In the decades since stem cells were isolated, several distinct progenitor cell populations have been reported in the peripheral blood mononuclear cell (PBMC) fraction, including hematopoietic stem cells (HSCs),[72] endothelial progenitor cells (EPCs),[73] mesenchymal stem cells (MSCs),[74] osteoclast precursor cells,[75]hemato poietic osteoclast precursor cells,[76] and a population of circulating fibrocytes,[77] suggesting that CESs may possess the potential to differentiate into a multitude of mature functional cell types in specific microenvironments.

1.7 Identification of neurodegenerative factors using proteomics

Diagnosis necessitates a brain biopsy or necropsy sample, although many sporadic cases have a typical clinical picture. Treatments aimed at inhibiting the neurodegenerative processes only focus on symptom management[78] and likely to be most effective if the treatment is initiated as early as possible. In recent years, there has been a growing interest in applying proteomics to study on clinical diagnostics and predictive medicine for neurodegenerative disorders and to discover proteins that are associated with pathogenic mechanisms for use as disease biomarkers.[79]

1.8 Two-dimensional electrophoresis analysis of whole cell proteomes and plasma proteomes

Human plasma is a rich source of biochemical products which can function as indicators of the physiological or clinical status of patients.[80] It also evidences high-protein content, including proteins that are synthesized and secreted, shed, or lost from body cells and tissues.[81] These protein concentrations in plasma are controlled tightly to balance their physiological functions in areas including immunity, coagulation, small molecule transport, inflammation, and lipid metabolism.[82] Therefore, changes in plasma protein concentrations can be considered reflective of the current state of health. Recently, the discovery of biomarkers from blood plasma has become the subject of intensive attention, with the considerable advances that have thus far been made in proteomic research.[83] The analysis of large numbers of proteins via proteomic techniques has greatly accelerated our ability to identify new biomarkers for disease or toxicity processes, including cancer,[84]diabetes,[85] stroke,[86] kidney diseases,[87] and exposure to aromatic hydrocarbons.[88]

The proteomes of cells are consisting of several thousand proteins. Because of this complexity, two-dimensional polyacrylamide gel electrophoresis (2DE) has been widely used as the standard protein separation and display method. Usually multiple samples are produced at different stages after stimulation, gene deletion or over-expression, or drug treatment experiments, and separated in a number of 2D gels. The scope of applications extends from drug discovery to diagnostics, therapy, microbiology, biochemistry, etc.

Two-dimensional gel electrophoresis separates proteins according to two independent parameters, isoelectric point (pI) in the first dimension and molecular mass (Mr) in the second dimension by coupling isoelectric focusing (IEF) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).[89] Proteins separated on 2D gels are visualized by either staining with Coomassie blue dye, silver stains, fluorescent dyes, immunological detection or by radiolabelling and quantifying using densitometers, fluoro- and/or phosphor imagers. Theoretically, 2DE is capable of resolving up to 10,000 proteins simultaneously, with approximately 2000 proteins being routine, and detecting and quantifying protein amounts of less than 1 ng per spot. The position of a spot in the 2D map is not the enough information for an exact identification of a protein. For routine analysis, protein spots of interest (e.g. up- or down-regulated proteins) are excised from the 2D gel, digested into fragments by specific proteases and then identified using mass spectrometry (MS) and database mining.[90]

As plasma contains such a variety of proteins in a wide and dynamic concentration range, two-dimensional gel electrophoresis (2DE) is currently the principal technique for the separation of such complex protein mixtures.[91]When applying 2DE to complex samples such as plasma for the discovery of biomarkers, it is critical to establish sample preparation procedures in order to obtain high-quality results. The most important component of this procedure is the method selected to (i)prepare plasma while minimizing the loss of plasma proteins, (ii) deplete high-abundant proteins, (iii) remove contaminants that may interfere with 2DE analysis, and (iv) solubilize the proteins to transform each of them into a physicochemical state suitable for 2DE analysis.

1.9 Objective

The aims of the study are:

1. To determination of circulating endogenous stem cells (CESs) potency in terms of cell kinetics, growth and differentiation in culture system: effect of age and sex of donors.
2. To investigate potency of the circulating endogenous stem cells (CESs) in *ex vivo* using 3D-nanoscaffold models.
3. To characterize the circulating endogenous stem cells (CESs) obtained from the degenerative disease patients before and at different time interval after treatment using Siamois polyphenols.