CHAPTER I

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 this work, we study rigorously isolated the PBSCs from 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. This study will provide determining the parameters of cells kinetics and 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.

1.1 Stem cell

Stem cells represent the building blocks of our bodies, functioning as the natural units of embryonic generation during development, and adult regeneration following tissue damage [12]. They are defined by two distinct characteristics: The ability to maintain

themselves through cell division, sometimes after long periods of inactivity (self-renewal), and the ability to give rise to more specialized cell types (differentiation) [13]. Based on the stage in development they are derived from, stem cells are broadly classified as embryonic, umbilical cord, and adult stem cells. Adult stem cells are categorized as multipotent stem cells because they can differentiate into cell types different from their tissue of origin. Embryonic stem cells are more versatile in that they can develop into a greater variety of tissues than can adult stem cells. However, the therapeutic use of embryonic stem cells is still controversial because of ethical concerns, as well as immunological incompatibilities and concerns about uncontrolled development of malignancies or teratomas from administered cells [14]. In contrast, the use of adult stem cells is free of such ethical concerns, and, because the use is autologous, there are no concerns regarding incompatibility and rejection [15].

A variety of adult stem cell sources including adipocytes [16], mesenchymal [17], hematopoietic stem cells [18] and peripheral blood stem cell (PBSCs) for therapeutic purposes is now a main research topic of many laboratories. 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.

Let consider the peripheral blood stem cells (PBSCs), it is proposed to be a suitable stem cell source for cell therapy, because of it's a viability and easily separation, unfortunately the interest of use in clinical level is quite limited. In fact, the PBSCs was collected from donors after pretreatment using chemotherapeutic agents, such as cyclophosphamide, and other cytostatic drugs or growth factors in particularly granulocyte colony-stimulating factor (G-CSF) by which enhancing a mobilization of the stem cell into peripheral blood circulation [19]. Then the stem cells were collected through the apheresis procedure [20]. However, risk factor of G-CSF can also act on neuronal cells as a neurotrophic factor. Indeed, its receptor is expressed by neurons in the brain and spinal cord. The action of G-CSF in the central nervous system is to induce neurogenesis, to increase the neuroplasticity and to counteract apoptosis [21, 22], and side effect to bone pain, headache, fatigue, nausea, myalgia, dizziness and 'flu'-like symptoms are frequent adverse events in stem cell mobilization [23]. A

relevant adverse side effect of PBSCs mobilization is spleen enlargement [24]. Moreover, the stem cell obtained by this mobilization procedure was exposed to cytotoxic agents that might cause cellular damages that can impairment the biochemistry and physiology.

1.2 Parameters influenced on potency of PBSCs

Many factors could influence the mobilization of peripheral blood stem cells. In this study, we investigated the variables influencing the mobilization of peripheral blood stem cells based on age, gender, blood group, and microenvironment the affects there characteristics have on the behavior of peripheral blood stem cells.

1.2.1 Stem cells and aging. Stem cell and aging is a complex process that involves every cell and organ in the body and that leads to the deterioration of many body functions over the lifespan of an individual. The reduced capacity to regenerate injured tissues or organs and an increased propensity to infections and cancers are probably the most prominent features of aging. Adult stem cells such as hematopoietic stem cells (HSC) or mesenchymal stem cells (MSC) assure lifelong regeneration of adult tissues (e.g. all different types of blood cells or bone, fat and cartilage, respectively). If the rejuvenating effect of stem cells was perfect, senescent cells could be replaced indefinitely [25].

1.2.2 Gender. Gender is a concept that distinguishes sex between male and female. In the medicine gender is the field of medicine that studies the biological and physiological differences between the human sexes and how it affects differences in disease. Medical research has mostly been conducted using the male body as the basis for clinical studies. Gender is a factor that affects the abilities of peripheral blood stem cells (PBSCs). In a previous study, It was reported on the relationship between gender and the effect it has on the mobilization of patients with hematologic malignancies who underwent Auto-PBSCT. The result of these studies showed a

relationship between sex and the mobilization of peripheral blood stem cells [26].

1.2.3 Blood groups. Blood groups are a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Transfusion medicine is a specialized branch of hematology that is concerned with the study of blood groups. ABO- blood group incompatibility is of minor importance for the stem cell transplantation [27]. An ABO blood group incompatibility occurs if the recipients and the donors' blood groups were not identical. A minor mismatch our occur when the graft contains anti-A and/or anti-B antibodies that are against the ABO blood group antigens on the recipient erythrocytes. A major mismatch occurs when the patient had anti-A and/or anti-B antibodies against ABO blood group antigens on the donor erythrocytes. Bidirectional mismatches are defined as when both a minor and major mismatch occurred, such as A to B or B to A [28]. In a previous study to showed the effects of ABO blood group on the *ex vivo* proliferation of umbilical cord hematopoietic stem cells O blood group. It was found that the final number of cells was consistently higher in all the micro-wells planted with $CD34^+$ cells [29].

1.2.4 Microenvironment. Cell fate decisions are based on a complex set of input signals from the surrounding cellular microenvironment. The micro environmental cues can be presented to the cell in the form of soluble factors, the substrate or extracellular matrix (ECM) proteins onto which the cell is adhered, direct cell–cell interactions, mechanical forces or shear, the spatial organization (i.e. two-dimensional (2D) or three-dimensional (3D) architecture) of the microenvironment, or other various stimuli present in the cell's immediate surroundings [30].

1.2.5 Extracellular matrix. Extracellular matrix (ECM) represents an essential player in stem cell niche, since it can directly or indirectly modulate the maintenance, proliferation, self-renewal and differentiation of stem cells. Several ECM molecules play regulatory functions for different types of stem cells, and based on its molecular composition the ECM can be deposited and finely tuned for providing the most appropriate niche for stem cells in the various tissues [31].

1.2.6 Scaffold. The extracellular matrix (ECM) represents the secreted product of the resident cells of each tissue and organ and thus logically defines the ideal substrate or scaffold for maintenance of tissue specific cell phenotype. Biologic scaffold materials composed of extracellular matrix (ECM) are typically produced by decellularization of mammalian**.** Tissues and have been shown to facilitate the functional reconstruction of several tissue types [32]. Tissue-engineering scaffolds play a pivotal role in cell seeding, proliferation, and new tissue formation in three dimensions. Biomimetic scaffolds have received considerable attention for tissue regeneration in recent years, because they mimic extracellular matrix (ECM) and may provide optimal physiological environment for cells [33].

1.2.7 Culture supplement. In this context the choice of tissue culture supplement is of critical importance [34]. Fetal bovine provides vital nutrients, attachment factors, toxin scavengers and growth factors [35]. In order to characterize further the consequences of different culture supplements, we assessed critical parameters of MSC biology during long-term culture in FBS and HS supplemented media, such as proliferation, cell cycle, changes in phenotypic and senescence-associated marker expression, anchorage independent growth suggestive of transformation, differentiation potential and immune suppressive capacities. In aggregate, we verified that ASC cultivated in HS show signs of replicative aging and impairment of differentiation but no signs of transformation after long-term expansion,

comparable with cells cultivated in FBS, indicating that HS can be used safely as an alternative to FBS [36].

1.3 Objective

The aims of the study are: To determination of PBSCs potency in terms of cell kinetics, growth and differentiation in culture system: effect of age and sex of donors.