CHAPTER I

INTRODUCTION

Three-dimensional (3D) models of tissues will be revolutionary to the forthcoming tissue-engineering research. Recent work has demonstrated that embryonic stem (ES) cells differentiated on 3D artificial extracellular matrices highly porous, tantalum-based scaffolds have significantly higher hematopoietic differentiation efficiency than those cultured under conventional two-dimensional (2D) tissue culture conditions. Compared to 2D tissue culture plates, cells differentiated on porous, 3D-scaffolds possessed significantly higher expression levels of extracellular matrix (ECM)-related genes, as well as genes that regulate cell growth, proliferation and differentiation(Liu, Lin et al. 2006). In addition, these differences in gene expression were more pronounced in 3D dynamic culture compared to 3D static culture. It was reported that the specific genes are either uniquely expressed under each condition or are quantitatively regulated, i.e. over expressed or inhibited by a specific culture environment. The study of appropriate biomaterials for fabricating the scaffold is an emerging field of research. Recently, our research group successfully constructed a 3D-nanofibrous scaffold of different dimensions made from polyvinylidene fluoride (PVDF) polymers.

In this work, we proposed to use the 3D-nanofibrous scaffold made from PVDF as an artificial extracellular matrix to construct tissue derived from peripheral blood stem cells (PBSCs). The growth and differentiation of stem cells in the scaffold will be characterized. This study will provide foundation data of tissue formation and cell to cell interaction in 3D-model. The microenvironments obtained from such kind of experiments should represent those which exist in the tissue of living systems.

1.1 Stem cells

Stem cells are cells that have specific characteristics including self-renewal and the ability to differentiate into a variety of specific cell types. They can be classified into 2 categories based on cell origin, embryonic stem cells (ESCs) and adult stem cells (Kiatpongsan 2006). Embryonic stem cells are derived from the inner cell mass of blastocyte and are able to differentiate into cells and tissues of all three germ layers (endoderm, mesoderm and ectoderm). ESCs have been using for the study of cell-based therapy. Adult stem cells are undifferentiated cells and usually reside in tissue. They are still in an undeveloped state but have a potential to differentiate into a specific cell type of tissue or organ such as myocyte, muscle cell and hematopoietic stem cell. Hematopoietic stem cells (HSCs) are cells that form all types of blood cells in the body. They are found in bone marrow, umbilical cord blood and the blood stream. HSCs can be differentiated into muscle cell, bone cell, brain cell and skin cell. Human peripheral bloods were simplest access to the human hematopoietic stem cell population. They are routinely used for transplant in leukemia patient. The human peripheral blood stem cell

is augmented into the circulation with granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), alone or combination with cytoreductive agents(Fukushima and Ohkawa 1995; Arslan and Moog 2007). Mobilized peripheral blood stem cells (PBSC) are increasingly used to reconstitute hematopoiesis after high-dose chemotherapy for treatment of hematopoietic cancers and solid tumors (Rowley, Yu et al. 2001; Devine 2002; Corso, Varettoni et al. 2005; Sagar, Chaib et al. 2007). Ex vivo expansion of PBSC can be used to reduce time for hematopoietic recovery, following stem cell transplantation (SCT) or to overcome limited availability of PBSC or poor mobilization. Since the last decade, studies focused on cytokines, stromal cells, and combination of these two conditions to optimize the experimental conditions for hematopoietic stem cell (HSC) expansion HSC express *CD34* as regulators of hematopoietic cell adhesion to stromal cells of the hematopoietic microenvironment. Therefore, *CD34*-antigen, an integral membrane glycoprotein is used to characterize HSCs (Tao, Wang et al. 2004). The cell surface glycoprotein CD34 is used as surrogate marker for hemapoietic stem cell (HSC). The number of $CD34^+$ cells infused correlates with the leucocyte and platelet recovery, and a threshold of 2-3 $\times$ 10^6 $CD34^+$ cells/kg body weight seems to be necessary for secure engrafment upon transplantation.

In bone marrow, mesenchymal stem cells (MSC) are an important element of the bone marrow hematopoietic microenvironment and can differentiate into various stromal cells of the mesenchymal origin. In this microenvironment, MSC contribute to supply an appropriate scaffold for hematopoiesis and a complex network of cytokines, adhesion

molecules, and extracellular matrix proteins that regulate survival, proliferation, growth, and differentiation of HSC.

Characterization of stem and specific cells

As previously mentioned PBSCs possess potential to differentiate into various cells, if provided with the proper microenvironment. The long term culture of PBSCs will be used to mimic the *in vivo* situation for studying regeneration and organization of tissues. In culture conditions which are endogenous or exogenous of various cytokines and stimuli, will allow us to achieve the microenvironments and to regenerate cells of specific tissues at *in vivo* conditions. Thus, the mechanism underlying this observation of the co-existence of neurogenesis and angiogenesis might include the enhanced production of neurotrophic factors such as BDNF and PDGF by $CD34^+$ cell-derived neovasculatures and may directly result in differentiation of these $CD34^+$ stem cells into neuron/glial cells. Furthermore, $CD34^+$ cell-derived neovasculatures contributing to maintaining newly formed neuron/glial cells have been shown to integrate into networks in adult animals and provide an environment conducive to neurogenesis(Peterson 2004; Ding, Shyu et al. 2007).

1.2 Microenvironment

Interactions of cell can be classified into three types: cell-cell, cell-extracellular matrix, and cell-growth factor, each of which is functionally for both mature and developing cells. Some cell-cell interactions occur when lymphocytes interact with antigen-presenting cells to sites of tissue inflammation. For cell-extracellular matrix that

matrix component are important in the growth and development of precursor cell (Long, Briddell et al. 1992).

1.3 Extracellular matrix (ECM)

ECM is the extracellular part of mammalian tissue for structural support in addition to performing other functions. They have functions in cell adhesion, cell migration and cell proliferation. Components of ECM have two main classes of extracellular macromolecules which make up the matrix: (1) polysaccharide chains of the class called *glycosaminoglycans* (*GAGs*), which are usually found covalently linked to protein in the form of *proteoglycans,* such as heparan sulfate, chondroition sulfate or keratan sulfate, and (2) fibrous proteins, including *collagen, elastin, fibronectin,* and *laminin,* which have both structural and adhesive functions. ECM components significantly influence the growth characteristics of cardiomyocytes, development of spontaneous contractile activity and morphologic differentiation (Baharvand, Azarnia et al. 2005).

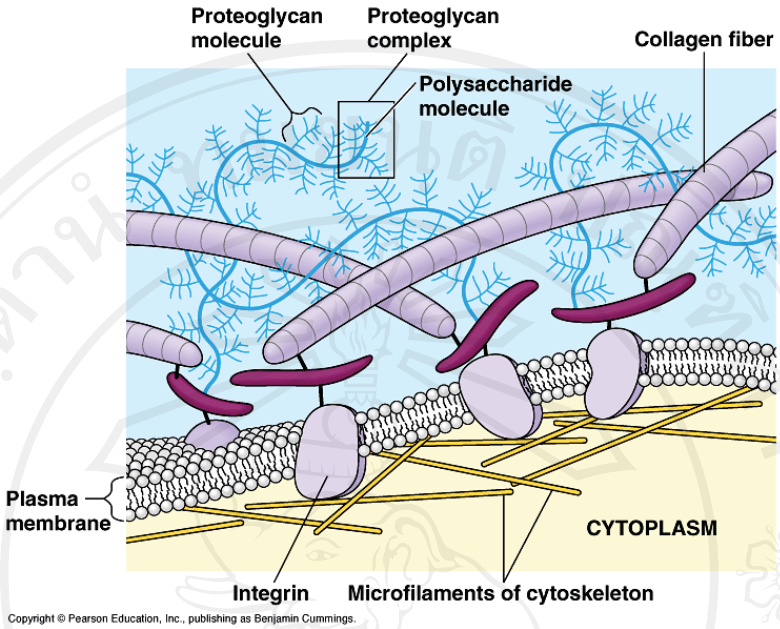

Figure 1.1 Structure of Extracellular matrix

1.4 Scaffold

3-D structures provide an ideal platform for cell–cell and cell–material communications and their properties can be varied to promote differentiation of cells into specific lineages. Scaffolds for tissue engineering serve numerous functions and their role during tissue development is dependent upon specific properties of the chosen biomaterial. 3-D systems have proven to enhance osteogenic, hematopoietic, neural, and chondrogenic differentiation. Scaffold, the so-called artificial extracellular matrix plays an important role in tissue repair and tissue engineering. The ideal scaffold should be made of: (1) biomaterial, and be biocompatible and biodegradable, (2) as well as be highly porous and have efficiency in the exchange of nutrients and waste-products between scaffold and the microenvironment. They should also be (3) resistant to stress and strain and hold good mechanical properties, and (4) be clinically compliant (Guillot,

Cui et al. 2007). Scaffold affects cell adhesion, cell migration and cell proliferation as has been reported. Thus, the study of the materials used to fabricate the scaffold is an emerging field of research. Both natural materials, such as gelatin, collagen, fibrinogen, and synthetic materials, such as polymers, ceramics and metals were used (Dawson, Mapili et al. 2008). Natural materials have advantage for being biological signal, biodegradation, biocompatible, and they have mechanical properties as native tissue. Disadvantage of them were weak mechanical strength, responded to immunogenetic, hard to modify, difficult to modify biodegradation rate and difficult to sterilization and purification of pathogen/virus (Hwang, Varghese et al. 2008). Native ECM such as collagen was used in 3D collagen gel has been to support ES cell-derived endothelial cells and neurite outgrowth. Synthetic material scaffolds were easy to control mechanical strength, degradation profile and porosity.

Poly(D,L)-lactide-co-glycolide(PLGA) scaffold has potential degradable biomaterials for dermal replacement (Blackwood, McKean et al. 2008). PLGA blended with collagen I and coated with E-selectin has increased efficiency of bone marrow-derived hematopoietic stem cell capture (Ma, Chan et al. 2008).

Polyvinylidene fluoride (PVDF) and its copolymer have been widely used for making ultrafiltration and microfiltration membranes owing to its excellent chemical resistance and good thermal stability. By using PVDF homopolymer, it is possible to obtain electric conductivities as high as 10^{-3} S/cm at room temperature, while outstanding mechanical properties are maintained. Highly porous PVDF matrices can be easily prepared by means of the phase inversion technique (Choi, Lee et al. 2004), which

is a well-known method for obtaining membranes with controlled and planned morphology. By means of this route, it is possible to prepare PVDF films which are able to absorb and retain a large amount of liquid electrolyte. PVDF could be used as the substrate for culturing neural stem cells, obtained from embryonic rat cerebral cortex, and cells exhibited different behavior (Hung, Lin et al. 2006).

OBJECTIVES

The aims of the study are:

1. To investigate the behavior and pluripotential of stem cells cultured in 3D-nanofibrous matrices.
2. To determine whether physical microenvironments in the matrices differentially affected the ability of stem cells reconstitution of cell communities and tissues.