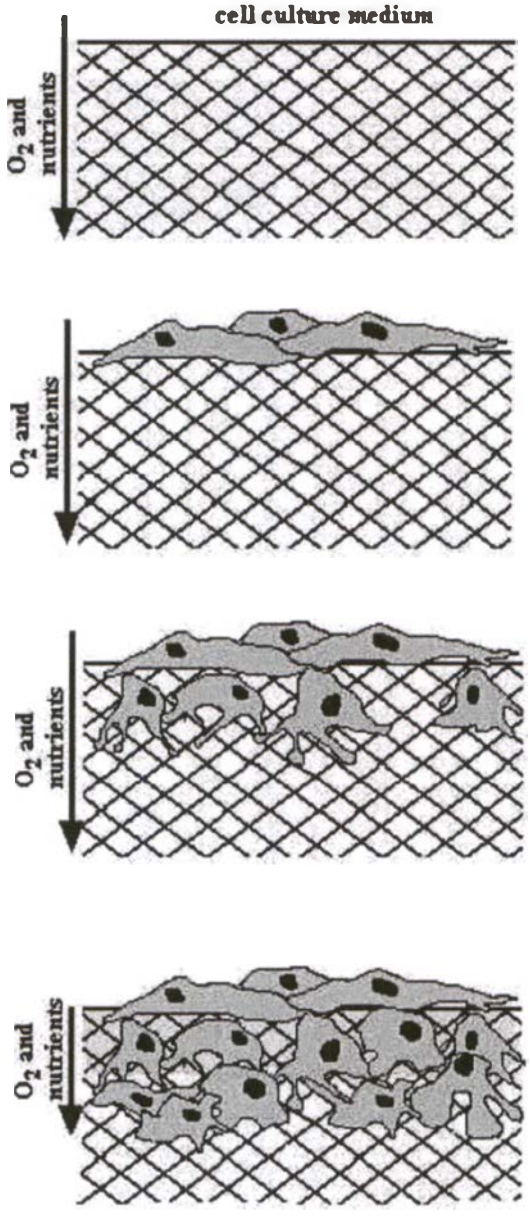
CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Tissue Engineering

The definition of Tissue Engineering, as stated by Langer and Vacanti , is ''An interdisciplinary field of research that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ'' (Langer *et al.*, 1993). They also stated that "Understanding the principle of tissue growth, and applying this to produce functional replacement tissue for clinical use. A further description goes on to say that tissue engineering is the new approach to overcome the limitations of the existing therapies for the treatment of malfunctioning or lost organ". One of the goals of tissue engineering is to develop method to produce the biological substitutes that will restore, maintain or even improve tissue or organ function. Generally, biocompatible and biodegradable polymers are used in tissue engineering to allow the growth of the tissue surrounding the area of implantation and enable cell attachment, proliferation differentiation and maintenance of cell function.

Porous scaffold was developed as native tissue integrates and actively promotes or prevent desirable and undesirable physiological responses. It provides a pore for cell to attach, proliferate, differentiate and secrete an extra-cellular matrix, eventually leading to tissue formation (Figure 1). The appropriate scaffold structure is also possible to guide cells into forming a tissue of predetermined.



a) Tissue engineering scaffold which contain pores structure. Oxygen and nutrients are supplied from liquid cell culture medium.

b) Cell seeded on scaffold.

c) Cells start to proliferate and migrate into the pores of scaffold.

d) The cells completely place in the pores and start to lay down their extracellular matrix

Figure 2.1 Concept of cell growth on the scaffold (Sachlos *et al.*, 2003).

When tissue engineering principles are applied to create bone substitutes to enhance osseous healing, various cell types, scaffolding materials and growth factors could be considered. The tissue engineering research program for bone and cartilage has been classified into six phases (Hutmacher *et al.*, 2000):

I. Fabrication of bioresorbable scaffold
II. Seeding of the osteoblast/chondocytes populations into the polymeric scaffold in a petri dish
III. Growth of premature tissue in a dynamic environment (spinner flask)
IV. Growth of mature tissue in a physiologic environment
V. Surgical transplantation

2.1.1 Interaction Between Cell-Protein-Biomaterial in Tissue Engineering

Extracellular matrix (ECM) was the first study on the adhesive interaction between cells and biomaterials. Interaction between living cells and foreign material is impossible. However, they can interact through a media absorbed to the exterior, proteins. These proteins can be specifically called as soluble matrix proteins in the biological fluids. Common examples of these proteins include fibronectin (FB), vitronectin (VN) and fibrinogen (FG). Not only the proteins are attached, but after a period of time, other ECM proteins (e.g. collagens and laminins) may accumulate and enhance the cellular interaction. The main factors that affect the biofunctionality and the biological response of a substrate are the concentration, distribution, and mobility of the adsorbed protein layer. Integrins, a type of cell surface receptor, is responsible for helping the cell recognize the protein matrixes by trans-membrane links between the ECM and the actin cytoskeleton. The integrins form clusters and provide focal adhesions to lead the cells to the material's surface and trigger the appropriate cellular response. They involve a team work of the receptor-ligand and the post-ligation interaction. Difunctionalities of the cell-ECM integrin are found to be due to pathologic cases, such as tumors. The mechanism of the interaction between the cell and the material is multi-

stepped, starting from adsorption of proteins to cell functioning. In order to study the parameters involved, it is wise to follow the classical approach, originally used as characterization of the cellular biocompatibility of materials (Salmerón-Sánchez *et al.*).

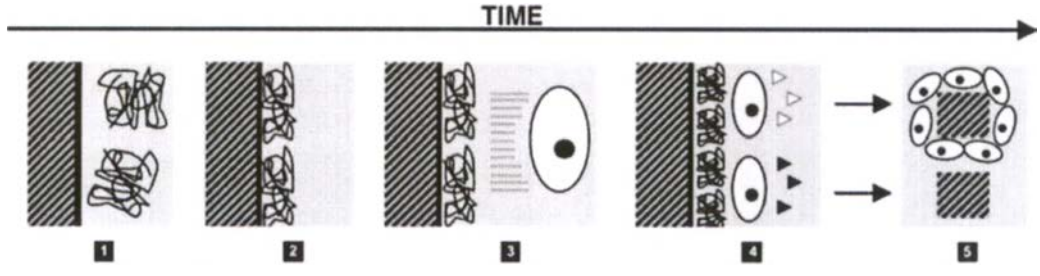


Figure 2.2 Schematic representation of the interaction between surfaces, proteins, and cells. Legend: proteins and surface before (1) and after (2) interacting; the proximal cells (3), by means of interacting with the surface/protein layer, initiate signaling mechanisms (4), which can lead in the end to a cell covering or to a cell-resistant surface (5). (Alves *et al.*).

Recent advances in biomaterial development have focused on the role of protein adsorption on cell attachment.

In 2001, Wyre and Downes coated fibronectin and vitronectin in PBS onto poly(ethyl methacrylate) and tetrahydrofurfuryl methacrylate (PEMA/THFMA) disc, which was prepared by mixing 5 g of PEMA powder and 3ml of THFM monomer liquid which contained 2.5% v/v N,N-dimethyl-p-toluidine (DMPT), to study their role in promoting cell attachment. The fibronectin improved attachment of cells but did not help cell to spread on the materials. Vitronectin was better and it was the main adhesive protein for chondrocyte attachment to TCPS and the PEMA/THFMA system in complete medium.

Six years later, Allen and his co-worker analyzed protein adsorption on the adhesion of HeLa cells onto the surface of N-isospropylacrylamide(NiPAAm):N-

tert-butylacrylamide (NtBAAm) co-polymer films tissue culture polystyrene (TCPS) with either albumin (5 mg/ml) or fibronectin (40 mg/ml). In summary, they found that the presence of serum proteins in the medium promoted cell adhesion to both TCPS and NiPAAm:NtBAAm co-polymers films, when compared with serum free protein medium.

Later on, Wang and his colleagues dissolved Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) in chloroform with a final concentration of 10 wt %, then the mixture was placed onto a glass substrate. After that RGD peptides were introduced on PHBV film through PEG-Containing Cross-Linkers to improve the biocompatibility. The results of cell cultured studies indicated that the RGD-modified films have more viable cells than the unmodified films.

2.1.1.1 Protein-Polymer Interaction

Protein adsorption can be defined as "adsorption (that is, adhesion or sticking) of protein(s) on a variety of surface" (Kim *et al.*) Proteins are the key factor that controls the interaction between the cell and the material, along with the characteristics of the system. This research will focus on the effect blending composition on adsorption of matrix proteins and the influence on cell response.

2.1.2 Scaffold Materials

2.1.2.1 Natural Polymers

Naturally derived protein or carbohydrate polymers have been used as scaffolds for the growth of several tissue types. By far, the most popular natural polymer used for tissue engineering scaffolds is collagen (Hayashi *et al.*, 1994).

2.1.2.2 Synthetic Polymers

Aliphatic polyesters such as polycaprolactone (PCL), poly(lactic acid) (PLA), poly(3-hydroxybutyric acid) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV) are the most commonly used polymers for tissue engineering

scaffold applications because of their biodegradability, biocompatibility, and bioresorbability (Bajgai *et al.*, 2008; Chung *et al.*, 2007). The degradation products of these polymers (glycolic acid and lactic acid) are present in the human body and are removed by natural metabolic pathways (Hayashi *et al.*, 1994).

2.1.2.2.1 Polycaprolactone (PCL)

PCL is a biodegradable polyester which is prepared by ring opening polymerization of ε-caprolactone. A melting temperature(T_m) and a glass temperature (T_g) of PCL are about 60°C and -60°C, respectively (Mattanavee *et al.*, 2009). PCL can be used as a part of wound dressing application, for example, degradable staple and in drug delivery devices because it is known as a nontoxic and tissue-compatible material which is approved by the US Food and Drug Administration (FDA). The structure of PCL is shown in Figure 3.

The degradation products, carbon dioxide and water, are endogenous compunds and they are non-toxic. Thus, PCL is considered for medical approaches, such as drug carriers, engineered skin, and scaffolds for supporting the growth of cells because of the presence of ester linakges in the PCL backbone allow the hydrolytic degradation of PCL.

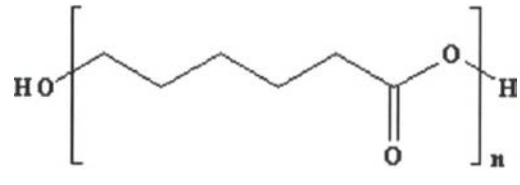


Figure 2.3 Chemical structure of Poly(caprolactone) PCL (Sangsanoh *et al.*, 2007).

2.1.2.2.2 Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV)

PHBV is one type of polyhydroxyalkanoate with a melting point temperatue (T_m) of about 153°C and a glass temperatue(T_g) of around 5°C (Fakiro *et al.*, 2007). It consists of copolymer between poly(3-hydroxybutyric acid) and poly(3-hydroxyvaleric acid). The structure of PHBV is shown in Figure 4. PBHV is

known to be biodegradable and biocompatible and its various properties such as natural origin, biodegradability, and biocompatibility make it suitable for variety of applications in health industry (Avella *et al.*, 2000).

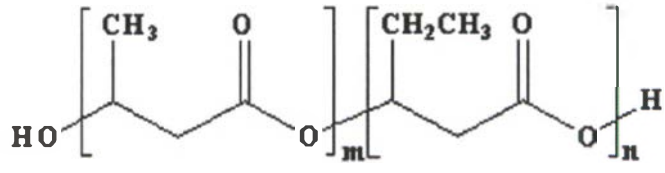


Figure 2.4 Chemical structure of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Sangsanoh *et al.*, 2007).

2.2 Polymer Blend

Polymer blend is a famous method that used to improve properties of polymer. By blending two types of polymers which are in fluid form, such as solution and molten, to be homogeneous. In addition, miscibility between the two components has tremendous influence on the morphology, thermal properties and mechanical properties for the blends. It is well known that binary polymer blends can usually be classified into three types in terms of the miscibility between the two components. They are completely miscible, partially miscible and completely immiscible polymer blends (Folkes *et al.*, 1993). The easiest method to study the miscibility of binary polymer blends is to investigate the glass transition temperature (T_g) of the blends if the difference in the T_g peaks of the two components is not less than 20°C. If polymer blends exhibit one single composition-dependent T_g peaks, the two components are completely miscible polymer blends. If polymer blends exhibit two composition-independent T_g peaks close to those of neat components, the two components are completely immiscible polymer blends. If polymer blends exhibit two composition-dependent T_g peaks which locate between those of neat components, the two

components are partially miscible polymer blends (Qiu *et al.*, 2005).

However, blending of two polymers rarely occurs naturally, making homogeneous polymers hard to come by. When the blended polymer solidifies, phase separation is often found. Phase separation is when one of the polymer components is in the continuous phase while the other component (discrete phase) is dispersed uniformly throughout the matrix. The heterogeneous phase could be caused by the difference in the polymer's chemical structure and polarity. In addition, another cause is due to the energy factor used for blending the polymer (Paul *et al* ,1988).

There were many research that study the blending of poly(hydroxybutyrate-co-hydroxyvalerate) and poly(e-caprolactone).

In 1999, Chun and Kim blended poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) and poly(caprolactone) (PCL) via solution casting by dissolving 0.6 g of the PHBV/PCL in 20 ml of chloroform at room temperature [3.0%(w/v) solution] for at least 1 day. Blends were casted on glass plates, and all film samples were dried under vacuum for 7 days at room temperature. They investigated thermal property and crystallinity of PHBV/PCL blended films with various composition by differential scanning calorimetry (DSC). From their results, the blends of PHBV and PCL were immiscible which is evaluated from the glass transition temperature(T_g) of the blend. Moreover, from the isothermal crystallization studies of PHBV in the PHBV/PCL blends, crystallization rate constant of PHBV in the PHBV/PCL blends decreased compared to that of the pure PHBV. From these results, it is concluded that the nucleation of PHBV in the blends is suppressed by the addition of PCL.

In 2005, Qiu and co-worker confirmed the results of Chun and Kim in 1999 that PHBV/PCL blended film was immiscible, which was investigated by differential scanning calorimeter(DSC) and optical microscope(OM) and the crystallization rate of blended decreased with the increase of PCL in the blends. In this study, the blended films were fabricated by preparing the solution of both polymers (0.02 g/ml) and then casting on a petri dish at room temperature. The solvent was allowed to evaporate in a controlled air stream for 1 day and the resulting films were further dried in vacuum at

50°C for 3 days. In this way, blends were prepared with various compositions ranging from 80/20–20/80 in weight ratio, the first number referring to PHBV.

Many researches showed that PHBV and PCL is immiscible and its effect to the degree of crystallinity and topology. From that point, topology from immiscible blends may turn out to be useful for improving the protein adsorption and influence the cell response.