CHAPTER II
LITERATURE REVIEW

2.1 Poly(caprolactone) (PCL)

Poly(caprolactone) can be synthesized from the ring-opening polymerization of ε-caprolactone. It is one of the biodegradable aliphatic polyesters which is widely used in the biomedical applications. The biodegradation occurs through the hydrolysis of its aliphatic ester linkage. It is a semicrystalline polymer with a low melting point (T_m) of 60°C and a glass transition temperature (T_g) of -60°C (Mattanavee, et al., 2009). PCL is currently being used as a part of wound dressing, degradable staple and in drug delivery devices because it is regarded as a nontoxic and tissue-compatible material which is approved by the US Food and Drug Administration (FDA). The drawback of PCL is its hydrophobicity that is unfavourable for cell adhesion and growth (Lin & Lu, 2002).

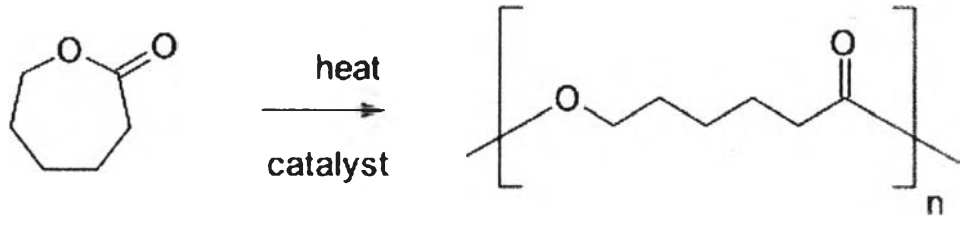


Figure 2.1 Structure of Poly(caprolactone).

2.2 Bovine Serum Albumin (BSA)

Bovine serum albumin is a globular protein with a molecular weight of 66,430 Da (Hirayama, 1990). BSA is a polypeptide chain that composed of 583 amino acid sequence. It is the most abundant protein found in plasma or circulatory system and has the pH ranges from 5-7. It has good water solubility. The important property of BSA is the ability to bind to variety of ligands, which makes it a carrier for fatty acid in circulating plasma.

2.3 Protein

Proteins are the building blocks of the cell and play roles in most of the cell functions. They can be found embedded in the cell membrane and also account for most of the cell's dry mass.

Protein is constructed from a long chain of amino acids. Each amino acid is linked to one another through a covalent bond called peptide bond. As a result, protein is also known as polypeptide. Amino acid in the chain determines the shape of protein. There are 20 types of amino acids found in protein. Different amino acid has different properties due to different side chains. Some side chains are hydrophobic (nonpolar) and the others are negatively, positively charged or have polar side group.

The long chain of protein can fold and be stabilized by many weak noncovalent bonds between different parts of the chain. Types of weak bond associated with protein folding are hydrogen bonds, ionic bonds and van der Waals attractions. Individual noncovalent bond alone is a lot weaker than covalent bond. However, many weak bonds can hold two region of polypeptide chain together.

Another weak force that affects the shape of protein folding is hydrophobic force. The amino acid with nonpolar side chain is considered hydrophobic. When the hydrophobic molecules are in aqueous environment, they tend to stay together to minimize the effect of hydrogen bond with water molecules. Therefore, the major factor contributing to the folding of the protein is governed by the distribution of polar and nonpolar amino acids. The amino acids with nonpolar or hydrophobic side chains tend to cluster together to form the hydrophobic core in the interior of molecule in order to avoid the contact with the surrounded water molecule. On the other hand, the amino acids with polar or hydrophilic side chains tend to arrange themselves outside the molecules so that they can form hydrogen bond with water molecules easily.

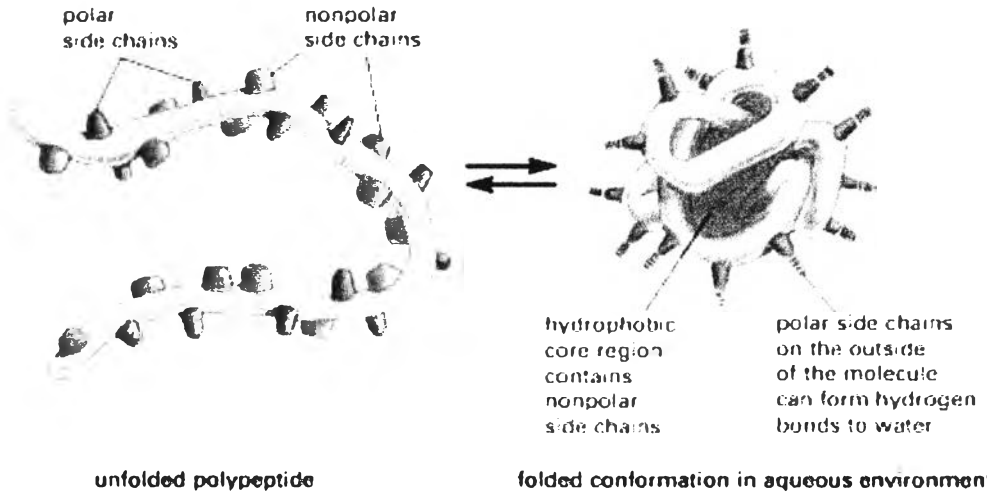


Figure 2.2 Protein folding.

Each protein has a specific three-dimensional structure. The final conformation is usually the one with the lowest free-energy. Upon the adsorption on the solid surface with different hydrophobicity, protein can undergo conformational change.

2.4 Protein Function

Each protein has its own amino acid sequence which determines its unique three-dimensional conformation. The particular protein shape allows it to bind to other selected molecules, for example, antibodies bind to viruses for destruction. The substance that binds to protein is called a ligand. The ability of protein to bind to a ligand depends on a set of noncovalent bonds. The position on the protein which binds to ligand is called binding site.

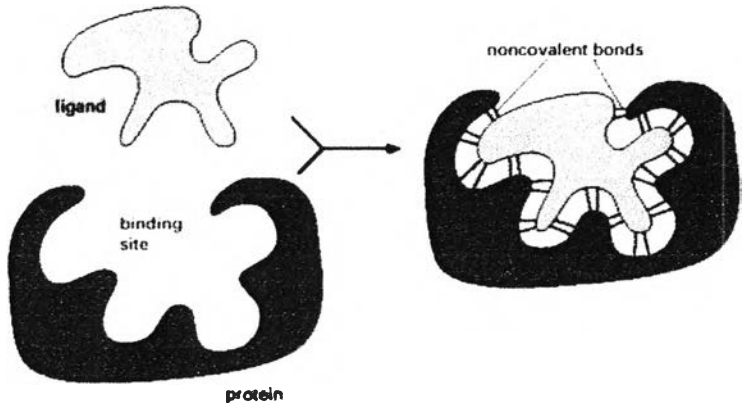


Figure 2.3 Protein binding site.

2.5 Cell Membrane

Cell membrane is important to the cell because it functions as a boundary that encloses the cell and maintains the difference between the cytosol and the environment outside the cell.

The common structure of biological membrane consists of the thin film of lipid and protein molecules. The lipid molecules arrange themselves in a form of continuous double layer called lipid bilayer which acts as a barrier to water-soluble substances. The membrane also has protein which performs other functions of the membrane such as transporting molecules across the membrane, sensing the external signal and allowing the cell to respond to its environment or acting as the receptor. Some proteins are the structural links which connect the cytoskeleton to the extracellular matrix. There are many different proteins to allow cell to function and interact with its environment.

There are two major components in biological membrane.

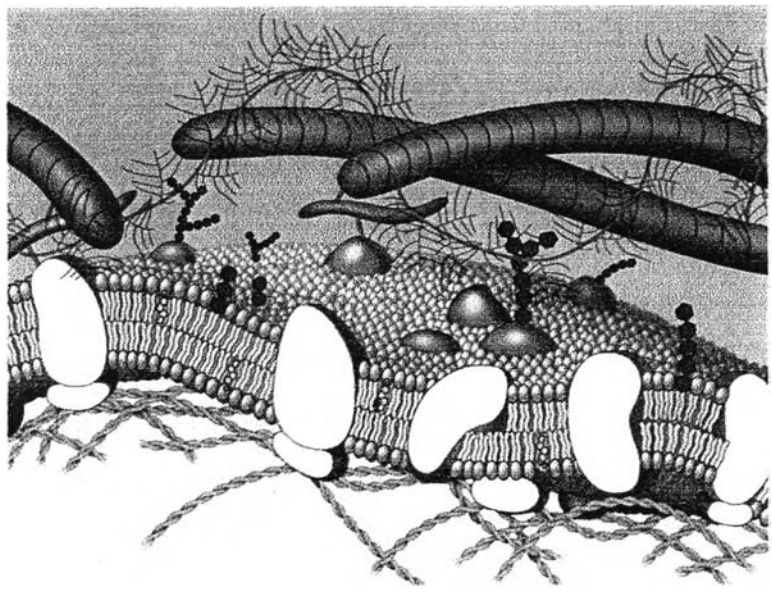


Figure 2.4 Cell membrane (http://learners.in.th/file/dawood/PlasmaMembrane.jpg).

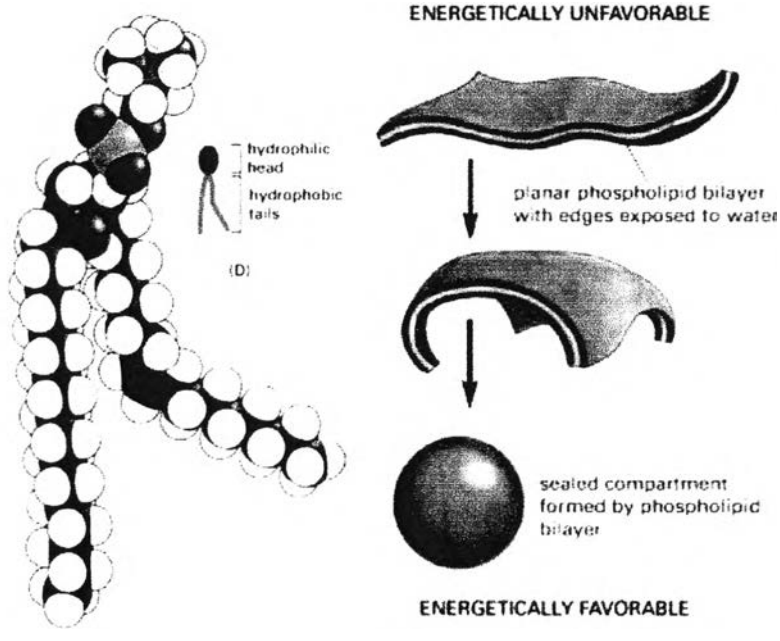
2.5.1 Lipid Bilayer

Lipid bilayer is a basis of cell membrane structure which made up for 50% of the total mass of the cell membrane. The rest belongs to the mass of proteins. Lipid is fatty molecules. All lipid molecules in cell membrane are amphiphilic, meaning that they have both hydrophilic (water-loving) end and hydrophobic (water-fearing) end.

The most abundant lipids in cell membrane are phospholipids. They have one hydrophilic head group with two hydrophobic hydrocarbon tails. In the aqueous environment, the lipid molecules spontaneously form into a bilayer structure due to its amphiphilic nature. Hydrophilic molecules contain charged group that can form hydrogen bond and dissolve in water. However, hydrophobic molecules have uncharged group that do not favorably interact with water molecule and so they are insoluble in water. When dispersed in water, hydrophobic molecules would form a cluster to reduce the number of interacted water molecules. With this reason, lipid molecules aggregate to hide the hydrophobic tails in the interior and expose their hydrophilic heads to water, forming the bilayer sheet in which the hydrophobic tails are sandwiched between hydrophilic heads.

It is not energetically favorable to have a tear or the free edge which exposed the hydrophobic molecules to water. In order to prevent this, the lipids

arrange themselves to form a sealed compartment in which the bilayer is closed on itself to eliminate the free edge.



a) b)

Figure 2.5 a) Structure of phospholipid b) The elimination of lipid-bilayer free edge.

2.5.2 Membrane Protein

Although the structure of cell membrane is determined by lipid bilayer, it is the membrane proteins that perform most of the functions. Proteins constitute for 50% of the membrane mass. Since lipid molecules are much smaller than the protein molecules, there are a lot more lipid molecules than protein molecules. There is approximately one protein molecule for every 50 lipid molecules for a membrane with 50% protein by mass.

The membrane proteins that extend through lipid bilayer are called transmembrane proteins. Transmembrane proteins are amphiphilic, having both hydrophobic and hydrophilic regions. The hydrophobic regions interact with hydrophobic tails of the lipids in the interior of the bilayer. The hydrophilic regions are exposed to water on either side of the membrane. Transmembrane proteins can function on both sides of the membrane.

Cell-surface receptor is one of the transmembrane proteins which can bind to the signal molecules in the extracellular region outside the cell and generate the intracellular signal in cytosol or inside the cell.

2.6 Cytoskeleton

Cells have to arrange themselves in space and response to their environment. They should be able to change their shape as they divide and grow. This adaptation is done under the filament system called cytoskeleton.

Cytoskeleton has many functions so it can be classified into three main types of filament; intermediate filaments, microtubules and actin filaments (Alberts, et al., 2002).

2.6.1 Intermediate Filaments

Intermediate filaments are ropelike fibers which extend across the cytoplasm to give cells their mechanical strength and resistance to shear stress. They are made from intermediate filament proteins. Each intermediate filament has a diameter of 10 nm.

2.6.2 Microtubules

Microtubules are long, hollow cylinders made from the protein called tubulin. With the diameter of 25 nm, it is more rigid than actin. The cylinders usually have one end attach to the center called microtubule-organizing center (MTOC) or centrosome. Microtubules determine the position of organelles and control intracellular transport.

2.6.3 Actin Filaments

Actin filaments are double helix molecule made from the protein called actin. The actin filaments have flexible structures. With the diameter of 5-9 nm, it is also known as microfilament. They can arrange into a network and are highly concentrate in the cortex beneath the plasma membrane. Actin filaments determine the shape of the cell and its motion.

The nucleation of actin filaments mostly occurs at the cell membrane. As a result, the actin filaments are found to be dense around the cell periphery. The

attachment of actin filaments to one another or to the cell membrane determines the movement and the cell surface. Actin structures can form different types of cell surface projection, for example, a spike-like shape (filopodia) or a flat sheet-like shape (lamellipodia). The actin nucleation is controlled by the external signal. This allows the cells to change its shape in respond to the environment.

However, these filaments are not effective on their own. They need the accessory protein to help linking them to other cell components to function.

2.7 Extracellular Matrix

Tissues are not made entirely from cells. There is a volume filled by a network of macromolecules such as proteins and polysaccharides called extracellular matrix. Extracellular matrix is the part of the tissue outside the cell. The network of secreted extracellular macromolecules has many functions. Extracellular matrix provides an organized environment and support that the cell can move and communicate with one another. Organism is made from different types of tissue.

In connective tissue, the cells are distributed within the matrix. The matrix has a lot of fibrous polymer such as collagen. It is also the matrix that bears the mechanical load on the tissue.

Extracellular matrix does not only function as the scaffold to stabilize the structure of the tissue but it also has a role in controlling cell behavior, proliferation and shape.

The macromolecules in the matrix are produced by the cell inside. Examples of macromolecules are proteins, including collagen, elastin and fibronectin.

2.7.1 Collagen

Collagen is a fibrous protein secreted by cells in connective tissue or other cell types. It is a main component in skin and bone. Collagen has a long, triple helix structure. It composes of three polypeptide chains or α chains wound around one another (Friess, 1998). The major amino acid found in collagen is proline and glycine. The ring structure of proline stabilizes the helical conformation of α chain. Glycine, as the smallest amino acid, allows three α chains to pack together closely.

Each individual collagen polypeptide chains are produced from ribosomes and then injected to endoplasmic reticulum (ER) as pro-α chains. Each pro-α chains are H-bonded together in endoplasmic reticulum to form triple helix molecule called procollagen. The propeptide cleavage at the end of procollagen molecule outside the cell by proteolytic enzyme converts procollagen into collagen molecules.

2.7.2 Elastin

Elastic fiber network in the extracellular matrix provides elasticity to the tissue when it is stretched. Elastin is the main component of elastic fiber. It is a protein which is rich in proline and glycine.

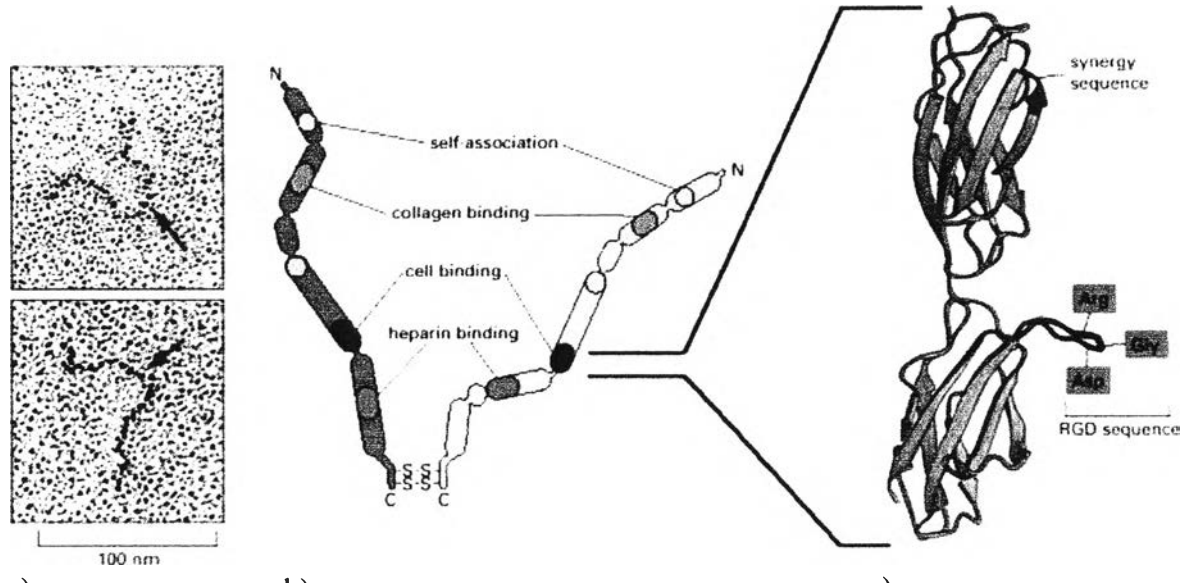
The precursor of elastin is known as tropoelastin. After tropoelastins are secreted, they cross-linked to one another to form a network of elastin fibers and sheets.

2.7.3 Fibronectin

There are other proteins apart from collagen found in extracellular matrix. Many of those proteins have multiple domains which function as specific binding site for other macromolecules in the matrix and for receptor on cell surface. These proteins help organizing the matrix as well as help attaching the cell onto it and they are known as fibronectin.

Fibronectin is a large glycoprotein. It is a dimer which has two large subunits that are connected together at one end by disulfide bond. Each subunit contains many domains that are separated by the flexible polypeptide chain. Each domain functions differently. Some domain binds to collagen molecules. Others such as cell-binding domain can bind to the receptor on the cell surface.

It is found that cell can bind to cell-binding domain because cell-binding domain contains the specific tripeptide sequence known as Arg-Gly-Asp or RGD sequence.



a) b) c)

Figure 2.6 a,b) Fibronectin structure c) The RGD sequence in fibronectin.

2.8 Arg-Gly-Asp or RGD Sequence

This short peptide sequence can bind to cell's binding site. Fibronectin is not the only protein containing the RGD sequence. This sequence can be found in many extracellular matrix proteins, for example, fibrinogen (blood-clotting factor).

The RGD sequence of the protein on extracellular matrix can help cell to bind to extracellular matrix because it can be recognized by a family of cell-surface receptors called integrin.

Although bovine serum albumin (BSA) itself does not contain integrin binding site, Ponik and coworker reported that osteoblasts which were cultured on BSA for 5 hours could secrete fibronectin or extracellular matrix proteins that contain binding site for focal adhesion (Ponik & Pavalko, 2004).

2.9 Integrin

For the cell to attach to the extracellular matrix, it requires a linkage or the transmembrane cell adhesion proteins to act as matrix receptor which tie the matrix to cell's cytoskeleton. The principal cell-surface receptor which binds cell to most extracellular matrix proteins, including collagen, fibronectin and laminin, is called integrin.

Integrin is the main receptor protein in which cell uses to bind and respond to extracellular matrix. Integrin composed of two subunits called α and β. The α and β subunits are held together by noncovalent bond.

Integrins function as transmembrane linkers, connecting between cytoskeleton (mostly actin) inside the cell and the extracellular matrix. After integrin binds to its ligand in the matrix, the tail of β subunit in the cytosol binds to intracellular anchor proteins such as talin, α-actinin and filamin. Theses anchor proteins bind directly to actin, linking the integrin to actin filament. The linkage could create the clustering of integrin to form the focal adhesion.

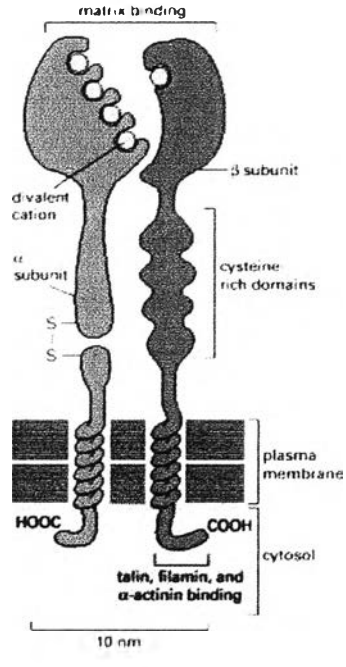


Figure 2.7 The structure of integrin.

2.10 Cell Junction

Cell junction is the point where cell and matrix is in contact with each other in all tissue.

2.10.1 Anchoring Junction

The lipid bilayer alone cannot provide enough force to attach cell to extracellular matrix. Anchoring junction mechanically helps cells and their

cytoskeletons to attach to extracellular matrix by forming a membrane-spanning structure that tie to actin filament inside the cell.

Anchoring junction is composed of two classes of protein: intracellular anchor protein and transmembrane adhesion protein.

2.10.2 Intracellular Anchor Protein

Intracellular anchor proteins are at the cytoplasmic side of cell membrane to link actin filaments to the junctional complex. Examples of intracellular anchor protein are talin, α-actinin, filamin and vinculin.

2.10.3 Transmembrane Adhesion Protein

Transmembrane adhesion proteins have a tail that can connect to intracellular anchor proteins inside the cell and the other part of transmembrane adhesion protein is on the outside of cell to connect to extracellular matrix. Example of transmembrane adhesion protein is integrin.

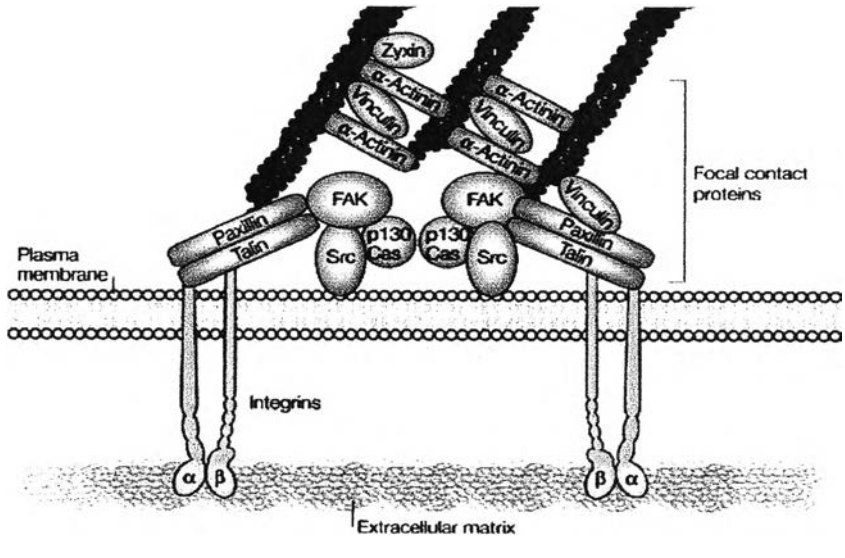


Figure 2.8 The focal adhesion complex (Mitra, Hanson, & Schlaepfer, 2005).

2.11 The Process of Cell Adhesion

2.11.1 Binding of Integrin to Extracellular Matrix Protein

Extracellular matrix proteins that contain RGD sequence such as fibronectin can be recognized by integrin which has the specific binding site for this tripeptide sequence.

2.11.2 Clustering of Integrin

The clustering by transmembrane adhesion protein of integrin can form a structure called focal adhesion. Focal adhesion binds cell to extracellular matrix and allow cell to pull on the substratum that it is bound to.

2.11.3 Focal Adhesion Links Intracellular Portion of Integrin to Actin Cytoskeleton

The function of actin cytoskeleton is to change the shape of the membrane. The effectiveness of the structure depends on the attachment of actin filament to the protein at the membrane. Actin helps connect the internal structure of a cell to extracellular matrix.

Focal adhesion allows cell to hold on to extracellular matrix via integrin that links intracellularly to actin filaments. When cells are cultured on a scaffold coated with extracellular matrix protein, they attach to substratum through focal adhesion where bundles of actin filament are. The extracellular domains of transmembrane adhesion protein, integrin, bind to extracellular matrix protein, while the intracellular domains bind indirectly to bundles of actin filament through the intracellular anchor proteins known as talin, α-actinin, filamin and vinculin.

2.11.4 Cell Proliferation on The Substrate

Focal contact does not only anchor cell to its substratum, but it also sends the signal from extracellular matrix to the inside of cell. The clustering of integrin generates focal adhesion kinase (FAK). FAK is sensitive to the type of substratum and can regulate the growth, proliferation, morphology and movement of cell in response to the extracellular environment by regulating the actin filament (Hall, Fu, Schaller, & Kwang).

2.12 Surface Modification of Poly(caprolactone)

Poly(caprolactone) is a FDA-approved biomaterial. It has good biocompatibility and biodegradability which make it desirable for biomedical applications such as tissue-engineering and drug delivery. However, poly(caprolactone) has a limitation that is a high hydrophobicity which is not suitable for cell growth and proliferation.

Cell adhesion and growth can be affected by the hydrophobicity or wettability of the surface that they attach to. There are many researches showing that cells prefer to attach and grow on a hydrophilic surface more than the hydrophobic one (Olah, et al.).

In order to improve the surface wettability, the modification is done by immobilizing the polar group onto the surface of poly(caprolactone).

2.12.1 Hydrolysis

The ester group (-COO-) on polyester can be hydrolyzed to carboxylic acid (-COOH) or hydroxyl group (-OH) under alkaline condition.

Gümüşderelioğlu et al. treated the poly(caprolactone) surface with NaOH to produce hydrophilic membrane. Their result showed that NaOH treated PCL had higher hydrophilicity than the neat PCL. This was proved by the decreasing in water contact angle from 64.3 ± 2.0^{o} in neat PCL to 31.2 ± 1.4^{o} in NaOH treated PCL (Gümüşderelioğlu, Kaya, & Beşkardeş, 2011).

2.12.2 Aminolysis

Aminolysis is the introduction of amino group onto polyester surface by diamine. An amino group at one end of diamine molecule can react with -COO- group of polyester to form a covalent linkage called amide bond, -CONH-. The remaining amino group at the other end of diamine can be used to connect to biomolecules.

Zhu et al. introduced the amino group onto poly(caprolactone) surface by the reaction of 1,6-hexanediamine and the ester group on PCL. The endothelial cell culture showed that the aminolyzed PCL surface slightly improved the cytocompatibility (Zhu, Gao, Liu, & Shen, 2002).

2.13 Immobilization of Biomolecules on Polyester Surface

Although polyesters are biocompatible, they are still not very appealing for the cells to attach to. In nature, cells bind to extracellular matrix because they sense the extracellular matrix protein. In order to make the synthetic scaffold more preferable to cell, the introduction of biomolecules is made. There are many biomolecules that are used to immobilized on substratum surface, for example, bovine serum albumin, collagen, gelatin, chitosan, fibronectin and RGD sequence-containing peptide (Mattanavee, et al., 2009; Peesan, Supaphol, & Rujiravanit, 2007; Sunami, Ito, Tanaka, Yamamoto, & Shimomura, 2006; Yu, Ying, & Jin, 2004; Zhu, et al., 2002).

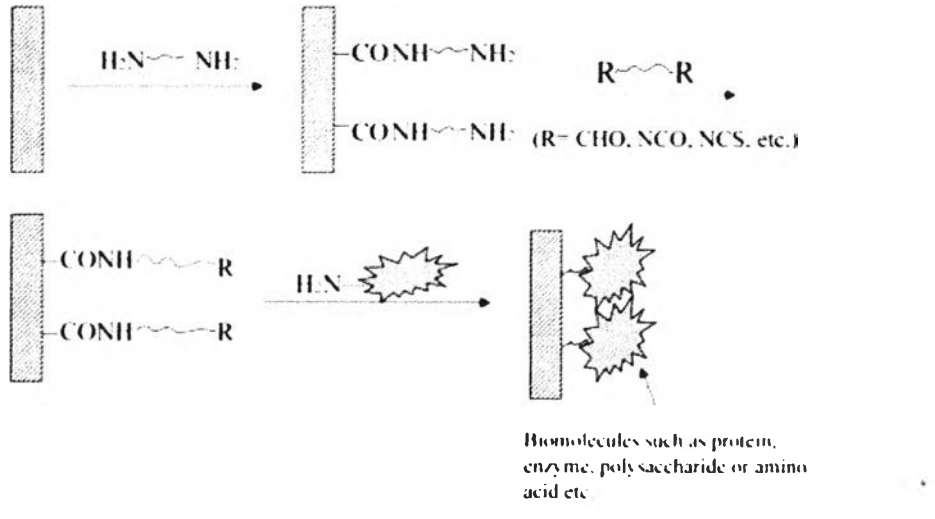


Figure 2.9 The aminolysis and immobilization of biomolecules on polyester surface.

After the aminolysis of PCL, Zhu et al. further immobilized biomolecules such as gelatin, chitosan and collagen. The result showed that after the immobilization of biomolecules, cell attachment and proliferation were clearly improved (Zhu, et al., 2002).

2.14 Semicrystalline Polymer

Semicrystalline polymers have both crystalline and amorphous regions. Crystallization in polymer can be found in polyamides, polyester and stereoregular vinyl polymers. Crystallization profoundly affects the physical and mechanical properties of the polymer. Moreover, crystallinity can lead to the formation of different surface topology (Gümüşderelioğlu, et al., 2011).

2.14.1 Effect of Heat Treatment on Crystallization

The processing variable can affect the thermal history of the materials. The thermal history determines how polymer chains line up and crystallize. After the processing and cooling of the polymer, the primary degree of crystallinity is set. However, the crystallinity can change. As the materials are subjected to heat, the chains slowly relax and the stress locking them is reduced. These molecular relaxations can be performed in the controlled manner by exposing the materials to the second heat-set treatment. This treatment is also known as annealing or secondary crystallization ("Effects of Conditioning Nylon Filaments,").

Annealing can be done by heating the materials in hot air oven. The annealing temperature is controlled to be higher than the glass transition temperature (T_g) where the molecules are allowed to move slightly, but lower than the melting point (T_m).

Once the chains are untwisted, they have more chance to align with the neighboring molecules and reorganized. As a result, annealing can give the materials higher degree of crystallinity.

2.15 Surface Topolography

Surface topography or surface roughness has an effect on cell behavior and growth.

2.15.1 Effect of Casting Solvent on Surface Topography

Many works have found that changing the solvent system in film casting could alter the surface characteristic of the membrane (Gümüşderelioğlu, et

al., 2011; Hongliang, 2004; Tang, et al., 2004). This could be explained by the difference in solubility parameter between the casting solvent and polymer.

2.15.1.2 Solubility Parameter

Solubility parameter (δ) is a numerical value that indicates the degree of interaction between materials (Bordes, et al.). Solubility parameter can be defined as:

$$\delta = \sqrt{\frac{\Delta E}{V}}$$

The term ΔE/V is the cohesive energy density where ΔE is the heat of vaporization and V is the volume. Cohesive energy density is the amount of energy required to remove a unit volume of molecules from their neighbors which, in turns, can reflect the strength of intermolecular interactions per unit volume. The conventional unit of solubility parameter is $(calories/cm^3)^{1/2}$.

For material to dissolve, it needs the same energy to separate the molecules from each other to be surrounded by the solvent. The square root of cohesive energy density could indicate about the solubility behavior.

The solvents with the similar value of solubility parameter as the polymer are regarded as a good solvent for that polymer. On the other hand, the solvents whose solubility parameters are very different from the polymer are referred to as poor solvent when poor solubility is observed (Hongliang, 2004).

In good solvent, the solvent molecules can interact well with the polymer chains and perform self-avoiding random walk where the polymer chains extend. However, in poor solvent condition, polymer chains have the self-intersecting random walk where the molecular chains cluster together in order to avoid the contact with the solvent, forming the phase separation.

Phase separation can cause the pore formation in polymer membrane upon the solvent evaporation during the film casting, making the film surface rougher. The higher the difference in solubility parameter between the solvent and the polymer, the rougher the surface of the membrane would be.

2.16 Protein Adsorption on Various Polymer Surface Topography

Membranes of different surface topography have captured the interest of biomedical applications. In 2002, Lin and coworker (Lin & Lu, 2002) showed that one way to create a porous poly(caprolactone) film with different porosity is by solvent-casting-leaching method. In this method, the pores in poly(caprolactone) membrane occurred by leaching out water-soluble polymeric porogen, poly(ethylene glycol) (PEG). By using various weight fraction of PEG to PCL, the porosity was varied. They found that the number of pores increased as they increased the initial loading of porogens (PEG). The result from DSC thermal analysis showed that the glass transition temperature (T_g), the melting temperature (T_m) and the heat of fusion of the PCL membrane did not depend on the amount of PEG, meaning that there was no interaction between PCL and PEG.

There are many other biocompatible materials used for scaffold application. Sangsanoh et al. (Sangsanoh, et al., 2007) investigated the biocompatibility of poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3hydroxyvalerate) (PHBV) and poly(caprolactone) (PCL). They reported that PHB, PHBV and PCL were nontoxic to mouse fibroblasts and human osteoblasts.

In 2002, Zhu et al. (Zhu, et al., 2002) introduced the amino group on the surface of poly(caprolactone) via aminolysis using the reaction of 1,6-hexanediamine and the ester group of PCL. After the aminolysis, the endothelial cell culture showed the slight improvement of cytocompatibility. The active NH_2 group was further used to immobilize biocompatible macromolecules such as gelatin, chitosan and collagen. After the macromolecule immobilization, the cell attachment and growth were greatly improved. The cytocompatibility was shown to be better when proteins were immobilized on the substrate.

Another biomaterial called poly(L-lactic acid) or PLLA was studied by Zhu et al. (Zhu, Gao, Liu, He, & Shen, 2004) in 2004 for its properties on endothelium regeneration after aminolysis and immobilization of biomacromolecules. The result showed that the surface NH_2 density increased with the increasing concentration of 1,6-hexanediamine and aminolyzing time. Also, the surface roughness increased

after aminolysis. The cell proliferation of both aminilyzed and biomacromolecule-immobilized PLLA films were proved to be better than the control PLLA.

Mattanavee et al. (Mattanavee, Supaphol, & Hoven) investigated the immobilization of biomolecules on PCL surface. The PCL film was aminolyzed by 1,6-hexanediamine (HMD). The aminolyzed PCL was immersed in *N,N'*-disuccinimidyl carbonate (DSC) to activate the aminolyzed PCL. The activated aminolyzed PCL was then transferred to collagen or chitosan solution to immobilize biomolecules on the surface. The water contact angles of the films were examined. It was reported that after aminolysis, water contact angle decreased from 85 °/50 ° in virgin PCL to 73 °/42 ° in aminolyzed PCL. After the activation of aminolyzed PCL by DSC, the water contact angle became 89 °/40 °. The surface of activated aminolyzed PCL was more hydrophobic showing that the hydrophilic amino groups were converted to hydrophobic succinimidyl ester group. After the immobilization with biomolecules, the surface became hydrophilic again. The water contact angles were 61 °/0 ° and 72 °/0 ° in collagen-immobilized and chitosan-immobitized PCL film, respectively.

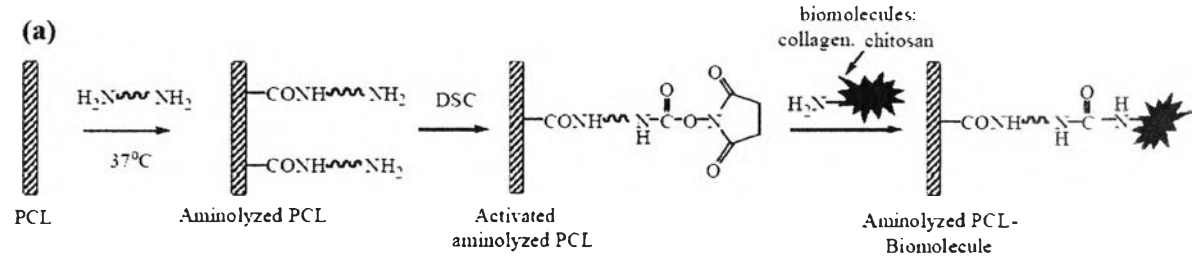


Figure 2.10 The aminolysis and biomolecule immobilization on PCL film (Mattanavee, et al.).

In 2004, Tang et al (Tang, et al., 2004). studied the surface properties of solvent-cast PCL films. PCL was dissolved in different solvent systems, chloroform, tetrahydrofuran, acetone and ethyl acetate. The SEM result showed that the surface morphology of the film casting from high dissolution solvent system (chloroform and tetrahydrofuran) gives the smooth aggregate structure while the film casting from the

less efficient solvent (acetone and ethyl acetate) gives the rougher surface and some filamentous structures.

Yu et al. (2004) (Yu, et al., 2004) studied the influence of hydrophobicity of silicon substrate on the adsorption of bovine serum albumin (BSA). The contact angle of hydrophilic silicon surface was 5 ± 1^{o} and for hydrophobic surface, it was 80 ± 1^{o}. It was found that BSA preferably adsorbed onto the hydrophobic surface. This was explained by the hydrophobic interaction that as the protein arrived on hydrophobic surface, it rearranged the structure in order to expose the inner hydrophobic group to the surface.

In 2006, Sunami et al. (Sunami, et al., 2006) examined the adsorption of a protein called fibronectin on the surface of honeycomb PCL film compared to a flat PCL film. Honeycomb film was a film with the formation of hexagonally-packed porous structure. After 24 hours of endothelial cell culture, cell number on honeycomb film was greater than the flat film. Moreover, the adsorbed protein was observed and fibronectin showed a site-selective adsorption behavior. They adsorbed on the inside of the pores. So, fibronectin in the flat film with no pore was hardly seen. The result showed that the cell number on honeycomb film was four times larger than flat film since focal adhesion points were mainly found inside the pore and endothelial cell adhered to the adsorped fibronectin.

In the same year, Yamamoto et al. (Yamamoto, et al., 2006) studied the effect of the fibronectin concentration on the amount of protein adsorped on honeycomb PCL film and on flat PCL film. Different fibronectin concentrations (0-1000 μg/ml) were adsorbed on the film for 24 hours. The amount of fibronectin adsorbed on both the flat and honeycomb films increased linearly with the incubation time and saturated after 1 hour. The adsorbed fibronectin on flat film increased with the increasing fibronectin concentration and then saturated when fibronectin-coating concentration was 600 μg/ml. The amount of adsorbed fibronectin on the surface of honeycomb film was found to be twice of the flat film due to the higher surface area of the honeycomb film.

In 2008, Arai et al. (Arai, Tanaka, Yamamoto, & Shimomura, 2008) investigated the effect of pore size of the fibronectin-immobilized PCL film to the

morphology and adhesion of cardiac myocytes. The films were prepared to have pore size ranging from 4 to 13 μm. Knowing that the size of cardiac myocyte was about 7 to 10 μm in diameter, the film pore size was grouped into sub-cellular size (4 μm), cellular size (8 μm) and over-cellular size (12 μm). The result of cell adhesion and proliferation was done by observing vinculin, a protein in focal adhesion complex. In the film with sub-cellular and cellular pore size, vinculin was faint after the first day of cell culture. It was after day 3 that vinculin clusters were clear and cells grew. However, for the film with overcellular pore size, vinculin were well-organized and did not depend on cell culture time.

In 2011, Gümüşderelioğlu et al. (Gümüşderelioğlu, et al., 2011) studied the effect of surface topography to fibroblast behavior. Surface topography may function as the anchor point of cells. They found that fibroblasts prefer rougher surface and higher porosity.