CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Tissue Engineering

Tissue engineering, also called regenerative medicine is an interdisciplinary field involving knowledge from medicine, biology, engineering and materials science fields. Tissue engineering makes use of scaffolds to provide support for cells to regenerate new extra cellular matrix which has been destroyed by disease, injury or congenital defects without stimulating any immune response. Natural extra cellular matrix (ECM) separates different tissues, forms a supportive meshwork around cells, and provides anchorage to the cells. It is made up of proteins and glycosaminoglycans (GAGs) which are carbohydrate polymers (Seema *et al.*, 2008)

Polymers have been widely used as biomaterials for the fabrication of medical device and tissue-engineering scaffolds (Nair *et al.*, 2007; Ghosh *et al.*, 2006) In biomedical applications, the criteria for selecting the materials as biomaterials are based on their material chemistry, molecular weight, solubility, shape and structure, hydrophilicity/hydrophobicity, lubricity, surface energy, water absorption degradation, and erosion mechanism. Polymeric scaffolds are drawing a great attention due to their unique properties such as high surface-to-volume ratio, high porosity with very small pore size, biodegradation, and mechanical property. They offer distinct advantages of biocompatibility, versatility of chemistry, and the biological properties which are significant in the application of tissue engineering and organ substitution.

Table 2.1 Ideal structural parameters of tissue engineering scaffolds (Edwards *et al.*, 2004)

Scaffold Functions	Scaffold Design Parameters
Not to activate inflammatory response or toxicity *in vivo.*	Must be biocompatible, non-toxic and noncarcinogenic.
To assist in the growth of three dimensional tissue and organs	Three dimensional scaffold of specific shape.
Give way to a uniform high cell seeding density	High porosity and high interconnectivity between pores.
To provide the appropriate surface for cell attachment, proliferation and differentiation of function.	Optimum polymer surface chemistry and topography.
To allow significant cell surface interactions such as cellular attachment.	High surface area to volume ratio.
To direct the orientation of cells, ECM and new tissue.	Correct fiber orientation within the scaffold.
To promote cell proliferation and migration leading to tissue growth throughout the scaffold.	Optimum pore size to allow for cell penetration, with high porosity and interconnectivity between pores.
To allow for the movement of nutrients and waste in and out of the scaffold.	High porosity and interconnectivity between pores.
The scaffold may degrade to leave only natural tissue.	Rate of degradation to match rate of tissue formation. Polymer degradation products must not be toxic or promote inflammation *in vivo.*
Possess sufficient structural integrity to retain shape *in vivo*, with enough mechanical strength to support developing tissue and withstand *in vivo* forces.	Scaffold should equal mechanical properties of developing tissue.

2.2 Polycaprolactone (PCL)

Polycaprolactone is a biodegradable thermoplastic polymer derived from the chemical synthesis of crude oil. Although not produced from renewable raw materials, it is fully biodegradable.

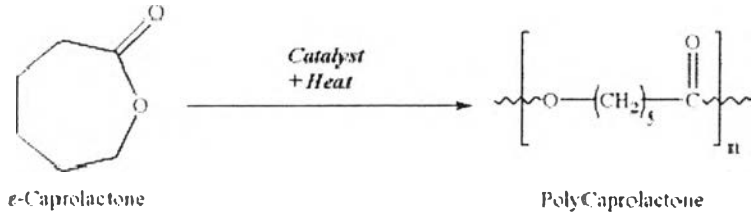

Figure 2.1 Structure of polycaprolactone (Labet *et al.*, 2009).

PCL is prepared by the ring-opening polymerization of the cyclic monomer ε-caprolactone and was studied as early as the 1930s (Van *et al.*, 1934)

PCL is a semi-crystalline polymer having a glass transition temperature (T_g) of −60 °C and melting point ranging between 59 and 64°C, dictated by the crystalline nature of PCL which enables easy formability at relatively low temperatures. The number average molecular weight of PCL samples may generally vary from 3000 to 80,000 g/mol and can be graded according to the molecular weight (Ulery *et al.*, 2011).

PCL is soluble in chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone and 2-nitropropane at room temperature. It has a low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide and acetonitrile and is insoluble in alcohol, petroleum ether and diethyl ether (Coulembier *et al.*, 2006).

2.3 Electrospinning Technique

The development of nanofibers has enhanced the scope for fabricating scaffolds that can potentially mimic the architecture of natural human tissue at the nanometer scale. Currently, there are three techniques available for the synthesis of

nanofibers: electrospinning, self-assembly, and phase separation. All of these, electrospinning is the most widely studied technique and also seems to exhibit the most promising results for tissue engineering applications. Nanofibers synthesized by self-assembly (Behravesh and Mikos, 2002) and phase separation (Zhang, *et al.*, 1999) have had relatively limited studies that explored their application as scaffolds for tissue engineering.

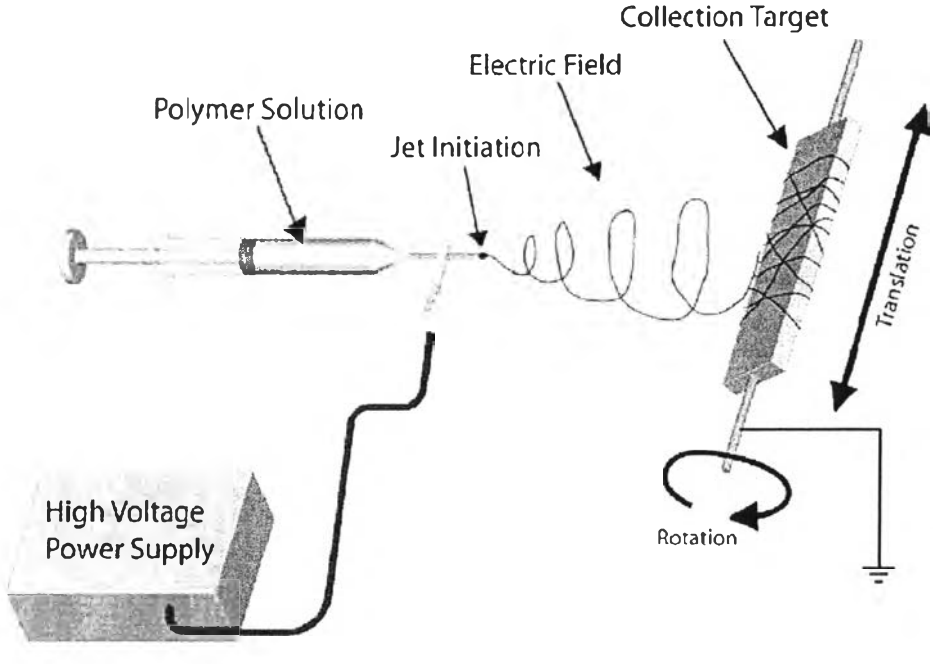

Figure 2.2 Typical electrospinning setup (Barnes *et al.*, 2007).

In the electrospinning process a high voltage is used to create an electrically charged jet of polymer solution or melt, which dries or solidifies to leave a polymer fiber (Frenot *et al.*, 2003). One electrode is placed into the spinning solution/melt and the other attached to a collector. Electric field is subjected to the end of a capillary tube that contains the polymer fluid held by its surface tension. This induces a charge on the surface of the liquid. Mutual charge repulsion causes a force directly opposite to the surface tension (Doshi *et al.*, 1995). As the intensity of the electric field is

increased, the hemispherical surface of the fluid at the tip of the capillary tube elongates to form a conical shape known as the Taylor cone. With increasing field, a critical value is attained when the repulsive electrostatic force overcomes the surface tension and a charged jet of fluid is ejected from the tip of the Taylor cone. The discharged polymer solution jet undergoes a whipping process (Rutledge *et al.*, 2002) wherein the solvent evaporates, leaving behind a charged polymer fiber, which lays itself randomly on a grounded collecting metal screen. In the case of the melt the discharged jet solidifies when it travels in the air and is collected on the grounded metal screen.

2.4 Biodegradable

PCL can be biodegraded by outdoor living organisms (bacteria and fungi), but they are not biodegradable in animal and human bodies because of the lack of suitable enzymes (Vert, 2009). That is not to say they are not bioresorbable, but rather, that the process takes much longer, propagating first via hydrolytic degradation. It is widely accepted that hydrolytic degradation of poly(α-hydroxy) esters can proceed via surface or bulk degradation pathways, depicted schematically in Fig. 2.3. The diffusion–reaction phenomenon determines the means by which this pathwayproceeds. Surface degradation or erosion involves the hydrolytic cleavage of the polymer backbone only at the surface (Ginde *et al.*, 1987).

From degradation studies presented in the literature it can be concluded that PCL undergoes a two-stage degradation process: first, the non-enzymatic hydrolytic cleavage of ester groups, and second, when the polymer is more highly crystalline and of low molecular weight (less than 3000) the polymer is showed to undergo intracellular degradation as evidenced by observation of PCL fragments uptake in phagosomes of macrophages and giant cells and within fibroblasts (Woodward *et al.*, 1985), which supports the theory that PCL may be completely resorbed and degraded via an intracellular mechanism once the molecular weight was reduced to 3000 or less. It was also noted that in the first stage the degradation rate of PCL is essentially identical to the in vitro hydrolysis at 40°C, and obeyed first-order kinetics. It was concluded that the mechanism of PCL degradation could be attributed to random

hydrolytic chain scission of the ester linkages, which caused a decrease in molecular weight.

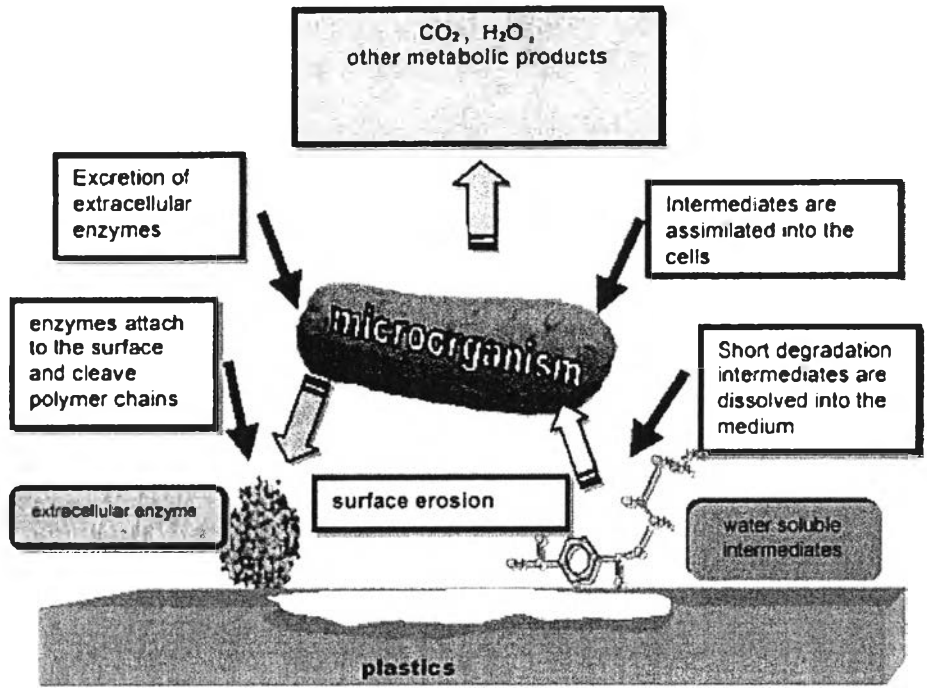

Figure 2.3 General mechanism of plastic biodegradation under aerobic conditions (Mueller *et al.*, 2006).

2.4.1 Enzymatic Degradation

Enzymes are biological catalysts, i.e., they accelerate the reaction rates in living organisms without undergoing themselves any permanent change. In fact, in the absence of enzymes, most of the reactions of cellular metabolism would not occur. Hydrolysis reactions may be catalyzed by enzymes known as hydrolases, which include proteases, esterases, glycosidases, and phosphatases, among others. This class of enzymes comprises cell-derived proteins that are responsible for the catalysis of several reactions in the human body. For example, hydrolytic enzymes are present in the plasma and interstitium, in the brush border membrane and lumen of the gastrointestinal tract, and in the tubular epithelium of the kidneys, where they

ensure the efficient hydrolysis of different substrates to facilitate absorption of nutrients and solutes.

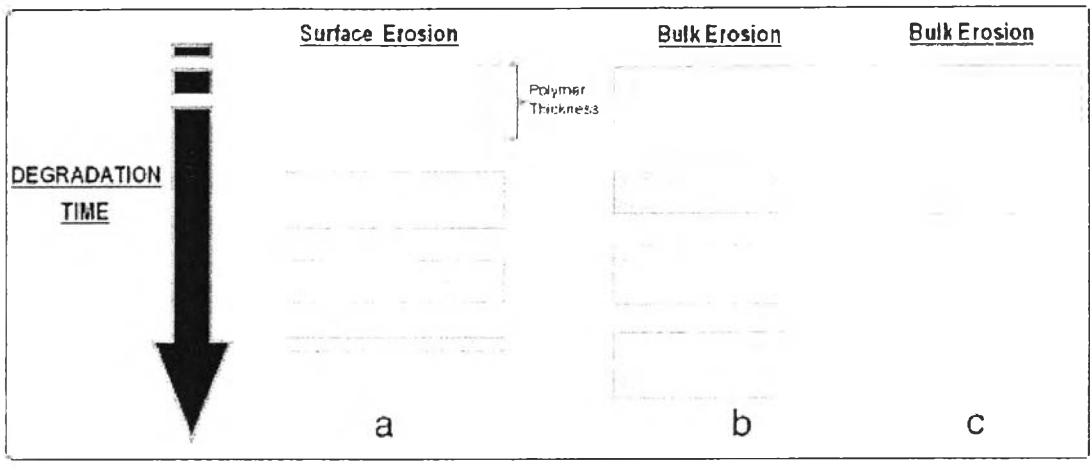

Figure 2.4 Schematic illustration of three types of erosion phenomenon: (a) surface erosion, (b) bulk erosion with autocatalysis and (c) bulk erosion without autocatalysis. (Vieira *et al.*, 2011)

2.4Importance of Biodegradability in Biomedical Applications

Biodegradable polymers have revolutionized the applications of biomaterial in the field of drug delivery and implants for tissue engineering applications. Scaffold degradation can occur through mechanisms that involve physical or chemical processes and/or biological processes that are mediated by biological agents, such as enzymes in tissue remodeling. The biodegradable scaffold gradually degrades by predetermined period to be replaced by newly grown tissue from the adhered cells (Langer *et al.*,1993) Degradation results in scaffold dismantling and material dissolution/resorption through the scaffolds bulk and/or surface types of degradation (Middleton *et al.*,2000) Polymeric scaffolds that undergo bulk degradation tend to break down the internal structure of the scaffold thus reducing the molecular mass (Woodruff *et al.*,2010). A polymeric scaffold that primarily undergoes surface degradation can be described similarly to the dissolution of soap. The rate at which the surface degrades is usually constant. Therefore, even though the size of the scaffold becomes smaller, the bulk structure is maintained. These

types of degrading scaffolds provide longer mechanical stability for the tissue to regenerate. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion (Katti *et al.,* 2002) The biodegradation rate of a polymer depends mainly on the intrinsic properties of the polymer, including the chemical structure, they presence of hydrolytically unstable bonds, the level of hydrophilicity/hydrophobicity, crystalline/amorphous morphology, glass transition temperatures (T_g), the copolymer ratio, and the molecular weight (Ye *et al.,* 1997) The Controllable degradation and restoration rates should match the rate of tissue growth *in vitro* and *in vivo* for biodegradable or restorable materials.

Once implanted, a scaffold material should maintain its mechanical property until it is no longer needed and then be absorbed and excreted by the body, leaving no trace. There are two types of biodegradation and both are discussed. A simple chemical hydrolysis of the hydrolytically unstable backbone is the prevailing mechanism for a polymer's degradation. This occurs in two phases. In the first phase water penetrates the bulk of the device, attacking the chemical bonds and converting long polymer chains into shorter water-soluble fragments. This occurs in the amorphous phase and initially there is a reduction in molecular weight without a loss in physical properties, since the device matrix is still held together by the crystalline regions. The reduction in molecular weight is followed by a reduction in physical properties, since the device matrix is still held together by the crystalline regions. The reduction in molecular weight is followed by a reduction in physical properties, as water begins to fragment the device. In the second phase, enzymatic attack and metabolization of the fragments occurs, resulting in a rapid loss of polymer mass. This type of degradation, where the rate at which water penetrates the device exceeds that at which the polymer is converted into water-soluble materials, is called bulk erosion. This results in erosion throughout the device. All commercially available synthetic devices and sutures degrade by bulk erosion. A second type of biodegradation, known as surface erosion, occurs when the rate of conversion of the polymer into water-soluble materials. Surface erosion results in the device thinning over time while maintaining its bulk integrity. In general, this process is referred to as bioerosion rather than biodegradation. In principle, the degradation rate of the

scaffold should match the rate of tissue formation. Therefore, the degradation behavior of a scaffold has crucial impact on the long-term performance of a tissue-engineeried cell/scaffold construct. Several factors, such as polymer molecular weight, polydispersity (Recum *et al.*, 1995), crystallinity (Pistner *et at.*, 1993), shape and morphology (Grizzi *et al.*, 1995), are known to affect the rate of hydrolytic degradation of polyester. Other factors, such as pH, ionic strength, temperature and buffering capacity of the medium in which the degradation occurs, also influence the degradation kinetics (Engineer *et al.*, 2011). Moreover, the chemical environment of the cleaved bonds, rigidity of the polymer chain, the molar mass of the polymer, adsorption and surface activation of the enzyme, removal and dissolution of fission products from the surface etc. are discussed to control the degradation process (Chandra *et al.*, 1998 and Scaherer *et al.,* 1999).