CHAPTER 1

Introduction

1.1 General Introduction

In recent years, biopolymers have been used in the biomedical field, such as tissue engineering, bio-artificial tissue, biosensor and drug delivery systems [1-10]. Biocompatibility is a crucial requirement for biopolymers to be used as biomaterials. However, many biopolymers do not contain intrinsic functional groups required for their specific applications [4, 11-13]. Poly (lactic acid) (PLA) is one of the best known biopolymers that has been used for several biomedical applications, such as surgical repair, carriers in drug delivery and temporary matrixes or scaffolds in tissue engineering (Fig. 1.1) due to its biocompatibility, and proper degradation rate which compared with the healing time of the human tissues [14-16]. Although PLA is widely used in biomedical fields, it has been reported that the hydrophobicity, the low surface energy and lack of recognition sites of cells of PLA greatly affect the cell attachment and proliferation [17-19]. For these reasons, the use of PLA in tissue engineering is limited since the cell attachment on PLA is rather low.

(a) (b)

Figure 1.1 Poly (lactic acid) in (a) the packaging industrials [20] and (b) biomedical applications [21].

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A number of studies have been performed on copolymerization, blending, stereochemical and processing manipulation of PLA to modify its performance for cell proliferation. These methods do not change, only the surface properties of PLA but also the bulk properties. However, the functional groups do not present in the backbone of PLA thus it is not easy to modify the surface of PLA by conventional chemical methods such as by attaching cell recognizing ligands to enhance cell attachment. Surface properties of PLA can be modified by non-permanent surface modification methods, such as coating, entrapment, migratory additives and plasma treatment [22-25], as well as permanent surface modification, such as chemical conjugation using wet chemistry and photografting [15, 26] to improved cell affinity for tissue engineering. Reactive groups such as carboxyl (-COOH), hydroxyl (-OH), and amine ($-NH_2$) as well as non-reactive groups, such as ether (-C-O-C-), alkene (-C-C-) and alkyl halide (-C-F) are typically introduced onto PLA using permanent or non-permanent surface modification strategies [27, 28].

Although plasma surface modification was considered to be a non-permanent method, this technique is convenient to induce the desired groups or chains onto the surface of a material. It has an advantage of treating surfaces of complex shape and it modifies only the top few nm of the surface and leaves the bulk properties of the material unchanged [29]. The surface modifications by plasma technique can be divided by functional groups as shown in Fig. 1.2. When a material is exposed to plasma, the interactions of the gas discharge with the surface are determined by the received flux of neutral particles, excited species, radicals and ions. These particles, especially the ions, may acquire large energies during passing through the plasma sheath. Depending on the chemical character of the species and their energy, one can observe different interaction processes with the surface [30]. The polymer modification depends on the energy input, which determines whether special chemical functions or highly cross-linked layers are obtained. Moreover, plasma process does not change only the surface compositions but also surface topography.

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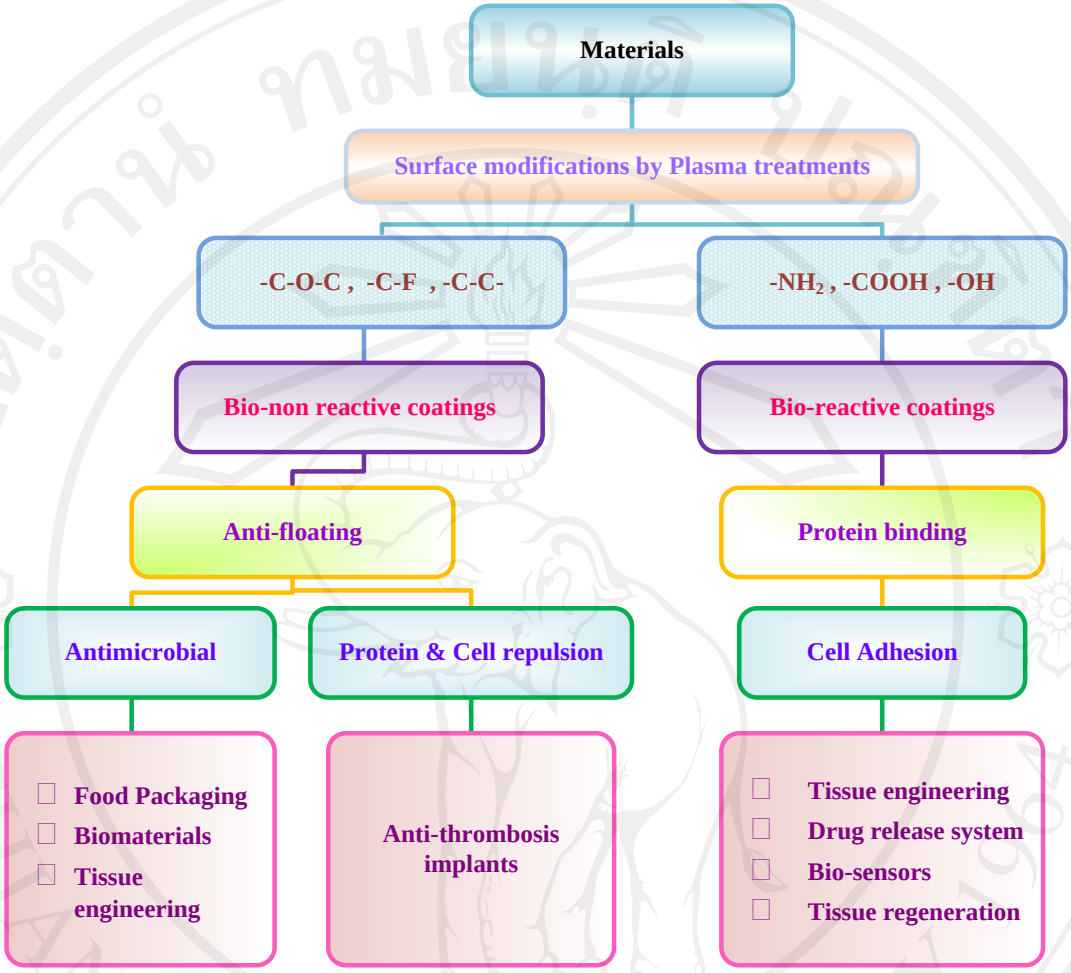

Figure 1.2 The surface modifications by plasma modifications divided by the surface functional groups.

1.2 Literature Review

As mentioned earlier, the proteins adsorption to a biomaterial surface is one of the important factors that determines the subsequent cell attachment, cell proliferation and therefore the biocompability of the material. A number of researchers studied the using of plasma technique to manipulate the surface properties of polymers variety that affected the cellular response.

Werner *et.al.* [31] studied on two human plasma proteins, human serum albumin (HSA) and fibrinogen (Fib) adsorption on hydrophobic fluorohydrocarbon

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polymer (CHF) films. There was no reversibility of the adsorption of the two globulins proteins on the surface. Coverage of the polymer surface to the adsorbed molecules was achieved by different amounts of HSA or Fib, depending on the transport conditions in the adsorption process. Structural of the protein layers were related to re-orientations and/or intramolecular changes of the adsorbed molecules. Results showed HSA and Fib adsorb irreversibly on the hydrophobic surface.

Previous studies have shown that oxygen, nitrogen and ammonia plasma can provide polar functional groups, such as C-O, C=O, -N-H-and -NH_2 [17, 32, 33]. Gao *et.al.* [34] modified polystyrene with Ar/O_2, He/O_2, N_2/H_2, and NH_3/H_2 plasmas. Ar/O_2 and He/O_2 plasmas are supposed to produce oxygen-containing functional groups while N_2/H_2 and NH_3/H_2 plasmas can produce nitrogen-containing functional groups at surfaces. It was found that NH_3 plasma was more effective to induce nitrogen-containing functional groups. Incorporation of oxygen-containing and nitrogen-containing groups could enhance the hydrophilicity of polymers. Yang and Lee [17, 33] reported that the positive charge groups on the surface of PLA was induced by O_2, N_2 and NH_3 plasma could enhanced the affinity of mouse fibroblast which were negatively charged cells. Wang *et.al.* [5] showed that H_2O and NH_3 plasma treatments improved the hydrophilicity and roughness of poly (butylenes succinate) (PBSu) due to the emergence of C-OH and C-NH_2 functional groups. Yanpeng *et.al.* [35] incorporated the amino groups onto the poly (L-lactic acid) PLLA surface to improve the hydrophilicity and biocompatibility. Zhao *et.al.* [36] enhanced the surface hydrophilicity of biodegradable poly (D,L-lactic acid) (PDLLA) films using nitrogen plasma and nitrogen/hydrogen plasma. Results indicated that the surface hydrophilicity of N_2 and N_2/H_2 was higher than untreated PLA.

Alves *et.al.* [37] reported that oxygen plasma treatment of a PDLLA increased oxygen-containing functional groups. The PDLLA films presented increased wettability, surface energy and water adhesion tension. The treated films improved bovine serum albumin (BSA) and fibronectin (Fn) adsorption from single protein solutions. Luensmann and Jones [38] reported that an increase in hydrophilicity of poly (2-hydroxyethyl methacrylate) (polyHEMA) lead to decreased HSA adsorption but

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promoted fibrinogen adsorption. Ying *et.al.* [39] showed that BSA was preferentially adsorbed onto a hydrophobic surface. They also investigated the competitive adsorption of albumin against collagen and found that albumin adsorbed onto the hydrophobic surface better than collagen. Although there have been many studies of protein adsorption onto surface of polymeric materials, it has not yet provided a complete picture. Han *et.al.* [40] studied an effect of helium atmospheric pressure glow discharge (He-APGD) treatment of titanium on selective protein adsorption and the initial attachment processes and focal adhesion formation of osteoprogenitor cells and stem cells were also examined. He-APGD treatment effectively modified the surfaces by creating a super-hydrophilic surface, which promoted selectively adsorption of fibronectin. The He-APGD treatment enhanced attachment cells area. Mathilde *et.al.* [41] investigated the MC3T3-E1 osteoblast-like cell behavior on silicon oxide and PLLA substrates. Using the adhesive protein fibronectin and the non-adhesive protein albumin adsorbed on the substrates. PLLA substrates coated with fibronectin and subsequently exposed to albumin exhibited the highest level of cell differentiation, as assayed via alkaline phosphatase activity.

The ultimate goal of this research is to design and fabrication of biomaterials with tailored properties. In order to achieve the ultimate goal, we divided it into 3 sub-objectives as follows: to modify the physicochemical of PLA surface using low-pressure plasma, to investigate the surface characteristics of the plasma treated PLA for understanding relationships between the material surface properties and protein adsorption and to study behaviors and preferential adsorption of different proteins on the plasma treated PLA surface.

In this work, we focus on the protein adsorption onto a PLA surface. We employed NH_3 plasma treatment to induce amino functional groups onto the surface of PLA. The NH_3 plasma was produced by means of an inductively coupled discharge technique. Optical emission spectroscopy (OES) was used to identify active plasma species and to determine the changes in their relative concentrations as the radio frequency (RF) powers were varied. Surface topography of the films was observed under an atomic force microscope (AFM). The contact angle and surface energy of PLA

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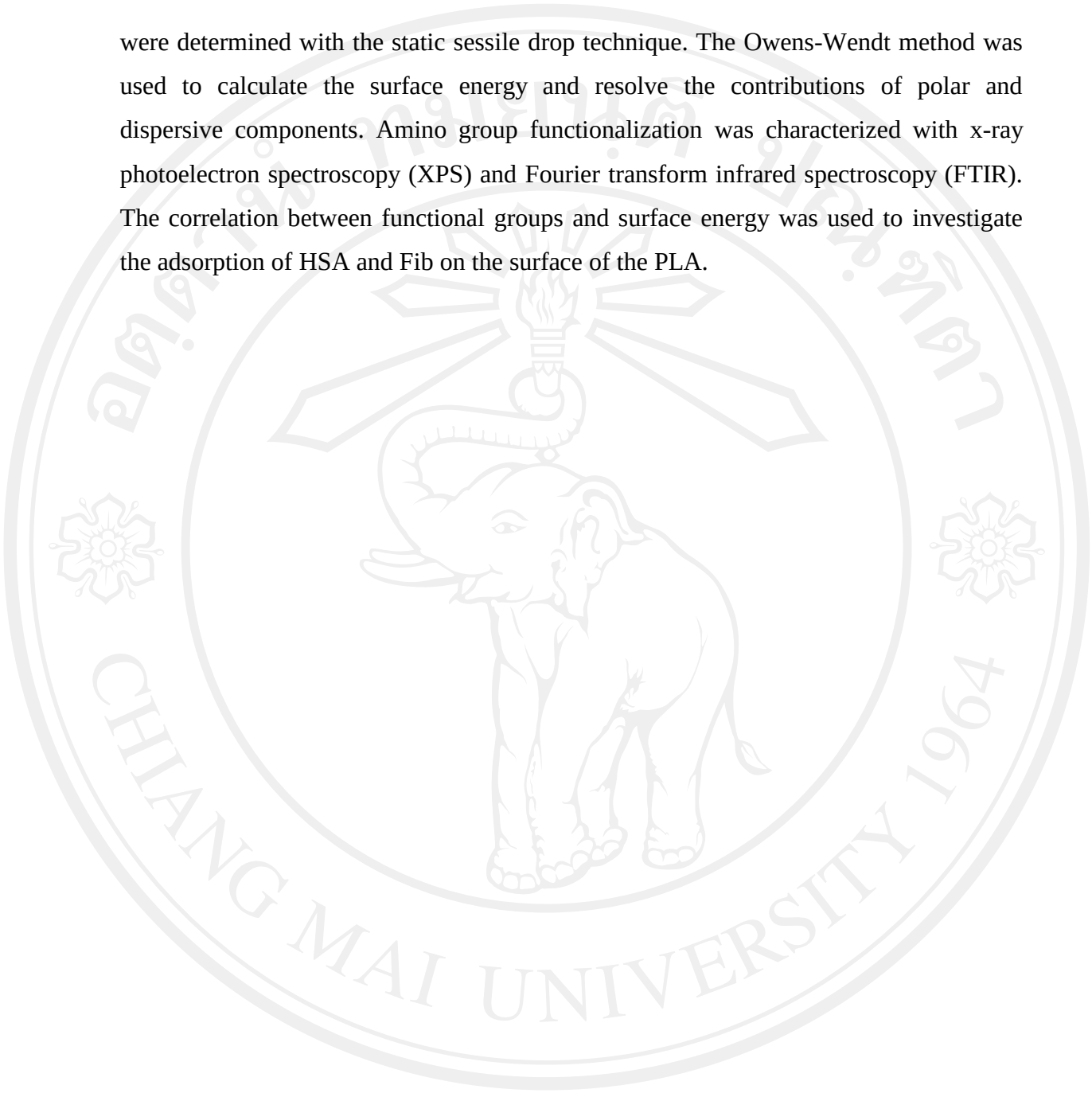
were determined with the static sessile drop technique. The Owens-Wendt method was used to calculate the surface energy and resolve the contributions of polar and dispersive components. Amino group functionalization was characterized with x-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). The correlation between functional groups and surface energy was used to investigate the adsorption of HSA and Fib on the surface of the PLA.



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