CHAPTER I
INTRODUCTION

Chitin or poly(*N*-acetyl-*D*-glucosamine) is one of the most abundant polysaccharides commonly found in shells of various insects and crustaceans as well as cell walls of various fungi. Chitosan or (1→4)-linked 2-amino-2-deoxy-*β*-*D*-glucan is a partially *N*-deacetylated derivative, obtained by hydrolysis, of chitin. It has structural characteristics similar to glycosaminoglycans (GAGs), such as condroitin sulfate and hyaluronic acid in the extracellular matrix (ECM) of human tissues. Among others, some potential uses of chitosan in biomedical applications are, for examples, tissue engineering scaffolds (Nettles, 2002; Li, 2005), wound dressing materials (Ishihara, 2002; Aoyagi,2006), and DNA (Lavertu, 2006) and drug (Lin, 2007) delivery systems. Despite chitosan has been explored as a suitable functional material for biomedical applications (Ma, 2001; Muzzarelli, 2001; Qi, 2004), mainly due to its biocompatibility with living tissue, biodegradability, and non-toxicity, its utilization is somewhat limited by its poor solubility and its physical properties i.e. rigidity and brittleness, a direct result of its strong intra- and inter-molecular hydrogen bondings.

Fortunately, chitosan is functionalizable due to appearance of hydroxyl and amino groups on its glucosamine units. Some chemical modifications such as phthaloylation (Nishimura, 1991; Kurita, 2001), alkylation (Yalpani, 1984), and acylation (Hirano, 1976; Zong, 2000) reactions were carried out to improve its solubility and to obtain desired properties. Therefore various derivatives of chitosan have been synthesized. These derivatives have much improved solubility and can be used to formulate by-designed materials for biomedical applications such as a tissue scaffolds (Seo, 2006), drug delivery systems(Tien, 2003), and wound dressing materials (Pielka, 2003) with high specificity and wide applicability. Among such derivatives, acylated chitosans are soluble in various common organic solvents, such as chloroform, benzene, pyridine, and tetrahydrofuran (THF) (Zong, 2000). With their excellent processability, N-acylated chitosans have been fabricated as membranes (Seo, 1995), films (Xu, 1996), and fibers (Hirano, 1998). Previous studies revealed some of their properties such as antibacterial activity, and blood

compatibility (Hu, 2007; Lee, 1995). The results from Lee *et al.* also showed that among various acylchitosans (i.e. N-propionyl, N-butyryl, N-pentanoyl, and N-hexanoyl), N-hexanoyl chitosan was found to exhibit the best blood compatibility (Lee, 1995). In addition, Hexanoyl chitosan (H-chitosan) was found to be anti-thrombogenic (Hirano, 1985). As a result, H-chitosan is a very interesting derivative of chitosan to be used in biomedical applications; however, its biological properties with living cells have not yet been reported. It is therefore one of the objectives of this work to investigate the biological properties of H-chitosan with mammalian cells.

In recent years, electrospinning process has attracted a great deal of attention due to its ability to produce ultrafine fibers with diameters in the range of nanometers to sub-micrometers and high surface area to volume or mass ratios (Doshi, 1995; Megelski 2002). The principle of electrospinning process is to use electrostatic force as the main driving force for fiber formation (Zhang, 2005; Shim, 2001; Theron, 2004). Morphology of the as-spun fibers depends on a number of parameters such as solution concentration, solution conductivity, applied electrostatic field strength, collection distance, collection time, feeding rate, and type of solvents as well as ambient temperature and humidity (Shim, 2001; Ding, 2002; Casper, 2004). Among others, some potential uses of electrospun fibers in medicine are, for examples, immobilization of enzyme (Wu, 2005), DNA (Luu, 2003) and drug delivery systems (Kenawy, 2002), wound dressing materials (Hong, 2006), and tissue engineering scaffolds (Rho, 2006; Suwantong, 2007; Wutticharoenmongkol, 2007). One of the obvious advantages of the electrospinning process over the film-casting and the conventional scaffold fabrication techniques is the three-dimensional with highly porous structures of electrospun fiber mats which exhibit much larger surface area that assumingly could allow drug molecules to diffuse out the matrix much more conveniently (Kenawy, 2002; Zong, 2002) and could better support attachment and proliferation of the cells cultured on the scaffold (Schindler, 2005; Moroni, 2008).

In this work, H-chitosan was synthesized via a heterogeneous acylation reaction between chitosan and caproyl chloride in chloroform/pyridine solvent. Films of H-chitosan were prepared by solution-casting technique and their compatibility

were evaluated through the indirect cytotoxicity study, and attachment , proliferation, and cell spreading study of L929, mouse connective tissue, fibroblast-like cells, on H-chitosan films *in vitro*. Morphology of the cells attached on the films was observed by scanning electron microscope (SEM). In addition, H-chitosan was electrospun into a mat composed of randomly oriented nano- and submicro-fibers. Parameters affecting the fiber size and morphology of the obtained as-electrospun fibers were studied. Since the nature of highly porous with three-dimensional fibrous structure that brings the electrospun mat to its possibility to be used as wound dressing or tissue scaffolds, the electrospun mat of H-chitosan was further evaluated for its biocompatibility with human fibroblasts and human keratinocytes, the two main cell types appear in skin tissues. The cytotoxicity study, cell attachment, and proliferation tests were carried out *in vitro*.