CHAPTER I
INTRODUCTION

Polymer blends have an advantage over neat polymers and copolymers that they are not limited by suitable synthetic schemes. Materials formed by mixing different polymers, therefore, become an appealing option, which is especially true for bio-functional polymers, as their chemical monomers are difficult to modify (Wang *et al.*, 2009). Poly(ε-caprolactone) (PCL) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), synthetic biodegradable polymers, have been utilized as biomaterials in medical fields such as orthopedics and oral surgery due to their biocompatibility and bioabsorbability (Niehaus, 2009; Tong, 2011). They are therefore suitable for further fabrication in to bone scaffolds. The blends of PCL or PHVB with another biocompatible and biodegradable polymer (e.g., polylactide (PLA) or polyethylene glycol (PEG)) have been shown to provide very good support for the attachment, proliferation, and differentiation of cultured bone cells (Fang, 2010; Chen, 2005). To improve both the mechanical and the osteoconductivity of the scaffolds, hydroxyapatite (HAp), a calcium phosphate ceramic that mimics the natural apatite composition of bone and teeth, is often used as a reinforcing bioactive agent (Wutticharoenmongkol *et al.*, 2007). Owing to the fact that the ideal artificial bone substitute should be both osteoconductive and osteoinductive, various bone proteins have been incorporated into the scaffolds to enhance the osteoinductivity (Wolfe *et al.*, 1999).

Electrospinning have been used for constructing fibrous tissue engineering scaffolds because the ultrafine fibers it produces mimic the nanofibrous structure of extracellular matrix (ECM) of human body tissues, which significantly enhances cell attachment and adhesion (Nisbet *et al.*, 2009). Herein, the incorporation of bone protein-loaded HAp nanoparticles into electrospun fibers to form fibrous polymer-HAp/protein composite scaffolds has been investigated. The morphology, mechanical integrity, and the physicochemical properties of all the obtained specimens were also studied. The biological evaluation *in vitro* of the specimens was subsequently conducted through cell culture experiments.

First, ovalbumin (OVA)-loaded HAp nanoparticles were synthesized at various pH conditions from two types of starting materials with different amounts of OVA initially added to find the optimal condition of OVA-loaded HAp nanoparticles, which could provide the optimal amount of OVA released. Bone proteins (e.g., type I collagen (COL), fibronectin (FN), and crude bone protein (CBP)) were then used as the loaded proteins within the HAp nanoparticles to generate HAp/COL, HAp/FN, and HAp/CBP composite nanoparticles. The release characteristics of the proteins from HAp/protein composite nanoparticles were also investigated.

Second, electrospun fibrous substrates with different surface topographies made from 50/50 w/w PCL and PHBV of varying concentrations, ranging from 4 to 14 wt%, was studied to analyze the effect of the morphology on bone cell behavior and to find the suitable concentration of the fibrous substrate that could show the best support for the osteoblastic activity.

Third, electrospun fibrous substrates from 10 wt% PCL/PHBV solution and 10 wt% PCL/PHBV with the presence of HAp, HAp/COL, HAp/FN, or HAp/CBP suspensions were prepared. The release kinetics of the three types of proteins from the fibrous substrates was also determined. The potential use of these fibrous substrates for bone regeneration was evaluated *in vitro* with mouse-calvaria-derived preosteoblastic cells (MC3T3-E1), in which the attachment, proliferation, alkaline phosphatase (ALP) activity, and mineralization were analyzed.

Ultimately, doxycycline hyclate (DOXY) was incorporated into PCL, PHBV, and PCL/PHBV blend (e.g., 75/25, 50/50, 25/75) solutions to prepare DOXY-loaded electrospun fibrous substrates for being used as drug carriers. The morphology as well as the structural and mechanical characterizations of all fibrous substrates was studied. The release behavior of DOXY from the DOXY-loaded fibrous substrates and *in vitro* antibacterial activity of those substrates were also examined.