CHAPTER I
INTRODUCTION

Electrostatic spinning or electrospinning is a process by which ultrafine fibers with diameters of the individual fibers in the range of sub-micrometer down to nanometer can be fabricated (Deitzel, 2001; Doshi, 1995). In this process, a continuous filament is drawn from a polymer solution or melt through a spinneret by high electrostatic forces and later deposited on a grounded conductive collector. Three main components for this technique are a high voltage power supply, a container for a polymer solution or melt with a small opening to be used as a nozzle, and a conductive collecting device. An emitting electrode of the power supply charges the polymer solution or melt by connecting the electrode to a conductive nozzle. Upon increasing the electrostatic field strength up to a critical value, charged species accumulated on the surface of a pendant drop destabilizes the partially-spherical into a conical shape (i.e. the Taylor cone). Beyond a critical value, a charged polymer jet is ejected from the apex of the cone and carried to the collector screen by the electrostatic force. The Coulombic repulsion force is responsible for the thinning of the charged jet during its flights to the collector. The charged jet elongates and, at the same time, dries out or solidifies to leave ultrafine fibers on the collector.

Due to high surface area to volume ratio of the electrospun (e-spun) fibers and high porosity in sub-micrometer length scale of the obtained non-woven mat, proposed applications for these materials are in areas such as filters (Gibson *et al.*, 1999), composite reinforcements (Bergshoef, 1999; Kim, 1999), carriers for delivery of drugs (Kenawy, 2002; Taepaiboon, 2006), and scaffolds for cell and tissue culture (Li, 2002; Teo, 2006; Meechaisue, 2006; Suwantong, 2007). The challenge in tissue engineering is the design of scaffolds that can mimic the structure and biological functions of the natural extracellular matrix (ECM) (Li, 2002; Teo, 2006). The important aspects of the e-spun fibers as tissue-engineered scaffolds are the three-dimensional structure with interconnected pores and high porosity that resembles the fibrous collagen in the natural ECM. The most commonly used biodegradable, synthetic polymers are polylactide (PLA), polyglycolide (PGA), polycaprolactone

(PCL), and their corresponding copolymers (Boyan, 1999; Hutmacher, 2000, 2001).

Osteoblast-like cells exhibited differing rates of mineralization when grown on different polymer surfaces (Calvert *et al.*, 2000, 2005). In their first report, Calvert *et al.* (2000) evaluated the ability of two common biodegradable, synthetic polyesters, i.e., PCL and poly (lactide-co-glycolide) (PLGA), as well as some of their blends in the form of thin films in supporting proliferation and differentiation of bone marrow stromal cells. They found that, at the end of 2 weeks after cell culture, while there was no statistical difference in the proliferation rate of the cells on any substrate, PCL was the only material that showed negative staining for alkaline phosphatase and calcification activities (Calvert *et al.*, 2000). Working on a different cell line (mouse calvaria-derived, pre-osteoblastic cells, MC3T3-E1), Calvert *el al.* (2005) showed in their subsequent report that, at 6 weeks after cell culture, while there was no statistical difference in the osteocalcin activity between the cells grown on PCL and PLGA films, PCL showed much less alkaline phosphatase activity and mineralization of the cells. However, PCL, due to its slow *in vivo* degradation (Bölgen *et al.*, 2005) is a good candidate for further fabrication into a bone scaffold. To improve both the mechanical properties and osteoconductivity of PCL scaffolds, rigid hydroxyapatite (HAp), a synthetic calcium phosphate ceramic that mimics the natural apatite composition of bone and teeth, is often used as a reinforcing bioactive agent (Kim, 2005; Chim, 2006).

In this work, the e-spun composite fiber mats of polycaprolactone (PCL; M_n = $8x10^4$ $g{\cdot}mol^{-1}$) with or without calcium carbonate ($CaCO_3$) or hydroxyapatite (HAp) nanoparticles (i.e. E-PCL, E-PCL/$CaCO_3$, and E-PCL/HAp) were prepared by electrospinning. HAp nanoparticles were synthesized by hydrolysis reaction following a method proposed by Shih *et al.* (2004). Morphological appearance and mechanical integrity of these e-spun fiber mats were characterized. Indirect cytotoxicity evaluation of the E-PCL, E-PCL/$CaCO_3$, and E-PCL/HAp based on human osteosarcoma cells (SaOS2) and mouse fibroblasts (L929) was investigated. The results suggested a high potential for use of these fiber mats as bone scaffolds, as they posed non-toxic to the cells. The potential use of the e-spun fiber mats as bone scaffolds was further evaluated *in vitro* with human osteosarcoma (SaOS2), mouse

calvaria-derived, pre-osteoblastic cells, MC3T3-E1, and human fetal osteoblasts (hFOB). For the study of SaOS2 cells, the E-PCL, E-PCL/$CaCO_3$, and E-PCL/HAp were evaluated in terms of attachment, proliferation, and alkaline phosphatase (ALP) activity of the cells that were cultured on the scaffolds in comparison with those on corresponding solution-cast film scaffolds and tissue-culture polystyrene plate (TCPS). For the study of MC3T3-E1 cells, the E-PCL and E-PCL/HAp were evaluated in terms of attachment, proliferation, differentiation, and mineralization of the cells that were cultured on the scaffolds. For differentiation study, the ALP activity as an early marker of differentiation as well as expression and synthesis of osteocalcin as a late marker of differentiation were investigated. The results were compared with those of TCPS. For the study of hFOB cells, the E-PCL and E-PCL/HAp were evaluated in terms of proliferation, ALP activity, and mineralization of the cells that were cultured on the scaffolds. Comparisons were made with the cells that were cultured on TCPS and porous poly(DL lactic-*co*-glycolic acid) (75:25) (PLGA) fabricated from solution casting and salt leaching technique.