CHAPTER I
INTRODUCTION

Silks are generally defined as protein polymers that are spun into fibers by some lepidoptera larvae such as silkworms, spiders, scorpions, mites and flies (Kaplan *et al.*, 1992, 1994, & 1998). Silk proteins are usually produced within specialized glands after biosynthesis in epithelial cells, followed by secretion into the lumen of these glands where the proteins are stored prior to spinning into fibers. Silks differ widely in composition, structure and properties depending on the specific source. The most extensively characterized silks are from the domesticated silkworm, *Bombyx mori*, and from spiders (*Nephila clavipes* and *Araneus diadematus*). The silk from the cocoon of *Bombyx mori* contains at least two major fibroin proteins, light and heavy chains, 25 and 325 kDa, respectively. These core fibers are encased in a sericin coat, a family of glue-like proteins that holds two fibroin fibers together to form the composite fibers of the cocoon case to protect the growing worm. Silkworm cocoon silk production, known as sericulture, produces high yields since the larvae can be maintained in high densities. Due to its strength, available in large supply and unique appearance, silkworm silk has been used commercially as textile production for centuries (Altman *et al.*, 2003).

In biomedical applications, silk in the degummed form (degumming is the process to remove sericin), i.e. silk fibroin has been used commercially as a medical suture for centuries (Vepari & Kaplan, 2007). This very long implantable use without immunogenic and inflammatory problems renders silk fibroin becoming the attractive material for broader use in biomedical applications. Now, many regenerated mophologies have been formed including films (Jin *et al.*, 2005), electrospun fibers (Unger *et al.*, 2004a,b), microspheres (Hino *et al.*, 2003; Wang *et al.*, 2007), and sponges (Li *et al.*, 2002; Kim *et al.*, 2005a,b) which mostly intended to be used as tissue-engineering scaffolds or drug-delivery carriers. Normally, the as-prepared regenerated silk fibroin materials have to be treated with a methanol solution to induce the trasition from the random coil conformation to β-sheet structure. The induction of the β-sheet formation is necessary for stabilizing the silk fibroin material in aqueous biological media. However, the β-sheet structure is also responsible for the

material's shrinkage, paritcular in the form of highly porous scaffold as well as for resistance to degradation (Jin *et al.*, 2005). Thus, to extend the biomaterial utility of silk fibroin, the degradation and dimensional stability have to be improved.

Chitin or poly(β(1-4)-N-acetyl-D-glucosamine) is one of the most abundant polysaccharides found in nature. It was found to have an accelerating effect on wound healing process (Mori *et al.*, 1997; Lloyd *et al.*, 1998). Due to the difficult dissolution in a common solvent, chitin was chemically modified to form a water-soluble derivative such as carboxymethyl chitin (CM-chitin). Ragnhild *et al.* (1997) showed that the biodegradability of this water-soluble polymer depended strongly on the degree of deacetylation (DD), the degree of substitution, and the substitution site. In addition to the chemical modification, chitin can be physical modified to form a nanoparticle, i.e. chitin whisker. This chitin whisker has been used as the filler for many nanocomposite systems to promote mechanical properties and thermal stability (Wu *et al.*, 2007) as well as to improve water resistance (Sriupayo *et al.*, 2005).

At another viewpoint, based on the advance in tissue engineering approach, the use of bioactive agents such as growth factors combined with a biomaterial in an appropriate microenvironment to help the body to heal itself has been a focus of attention. The key issue in enhancing regeneration of the injured tissue is seeking an appropriate scaffold to provide a niche for cell recruitment to complete functional restoration. A number of polymeric carriers with or without chemical modification, including collagen (Kanematsu *et al.*, 2004), gelatin (Tabata *et al.*, 1994, 1998, 1999a, 1999b), chitosan (Fujita *et al.*, 2005), hyaluronan (Pike *et al.*, 2006), fibrin derivatives (Sakiyama-Elbert & Hubbell, 2000), and poly(lactide-co-glycolide) (PLGA) (Shen *et al.*, 2008), are used to control the release of bioactive agents. Although these materials are biocompatible and provided sustainable delivery, they have the common disadvantage of poor mechanical properties. Silk fibroin, derived from silk of silkworm *Bombyx Mori*, is a very attractive scaffolding material and has the impressive mechanical properties, as compared to the commonly used polymers both natural and synthetic (Kim *et al.*, 2005b). Silk fibroin displayed less immunogenic and inflammatory, as compared to either collagens or PLGA (Meinel *et al.*, 2005). Thus, silk fibroin may be a good candiddate for controlled-delivery carrier in tissue engineering applications.

In this present dissertation, the development of silk fibroin-based biomaterials for tissue engineering applications was focused on two possible approaches, i.e. (i) improvement of the biological and physical functions of silk fibroin by blending/incorporating chitin derivatives, and (ii) the use of silk fibroin as a carrier matrix to delivery the bioactive agents. In the case of (i), in order to extend the utility of silk fibron, it was blending with chitin derivative, i.e. CM-chitin to imrpove its degradation. The protease was used as an enzyme for *in vitro* enzymatic degradation. In addition, incorporation of chitin whisker was expected to promote the dimensional stability of a freeze-dried silk fibroin scaffold. The mechanical properties, morphology and shrinkage of the prepared materials were charaterized. In the case of (ii), the study was conducted both *in vitro* and *in vivo* using dyes and basic fibroblast growth factor (bFGF) as low- and high-molecular weight model drugs, respectively. The affinity between model drugs and silk fibroin was also discussed. Additionally, the structure, morphology and biodegradation of the prepared carriers were investigated.