CHAPTER I
INTRODUCTION

The major problems of burn wound management and therapy are bacterial and fungal infections and wound sepsis, which may, in the worst cases, lead to mortality in patients. One approach for mitigating these problems is the use of antibacterial agents (Burkatovskaya *et al.*, 2006). Silver has long been recognized as a broad-spectrum and highly effective antimicrobial agent for treating infectious wounds (Klasen, 2000). Nowadays, the manufacturers of dressings such as Acticoat, Aquacel-Ag hydrofiber, Arglaes, Silverlon are used to enhance wound healing through the antimicrobial activity. However, these dressings are high cost. Therefore, we were interested to produce the antimicrobial wound dressing materials with low cost and high efficiency for using wound dressings.

Historically, silver has been used for numerous health care applications. The Greeks and Romans stored water in silver vessels to keep it fresh, and Americans traveling west during the 1800s put silver coins in water barrels to retard the growth of bacteria and algae (Russell and Hugo, 1994; Klasen, 2000). In 1884, a German obstetrician, C.S.F. Crede, formulated 1 % silver nitrate ($AgNO_3$) in eye drops to treat gonococcal ophthalmia neonatorum, which could be the first scientifically-documented medical use of a silver-based compound (Russell and Hugo, 1994). The use of silver-based dressings was shown to enhance epithelialization of clean wounds in pig models, indicating the beneficial effect of silver ions in wound care besides its antimicrobial activity (Geronemus *et al.*, 1979; Lansdown *et al.*, 1997). Laufman (1989) reported that 0.5% $AgNO_3$ aqueous solution may be as efficacious as antibiotics for the prophylaxis of burn infections. Notwithstanding, $AgNO_3$ is hypotonic and can seriously cause hyponatremia and hypochloremia (Poon and Burd, 2004; Vlachou *et al.*, 2007). Due to the reduction of silver ions, application of $AgNO_3$ can cause the color of the wound sites to change into dark gray or black, which can cause irritation (Lloyd and Hight, 1978; Qin, 2005; Hermans, 2006). To prevent these problems, much attention has been given to pure atomic silver, i.e., in the form of nanoparticles (hereafter, nAgs), which exhibits much stronger anti-microbial activity than the bulk silver metal (Cho *et al.*, 2005) due to their large

surface area to volume ratio (Lopez *et al.*, 2005). Their small size also provides greater particulate mobility and transdermal penetration (Suzuki *et al.*, 2001; Shahverdi *et al.*, 2007). The antibacterial activity of silver-based materials is generally attributed to four mechanisms. Ionic silver binds to the bacterial cell membrane (damaging it and/or interfering with various receptors); interferes with bacterial electron transport (impeding the production of adenosine triphosphate, the cell's energy "currency"); binds to bacterial DNA (impairing cell replication); and causes the intracellular formation of insoluble compounds with certain nucleotides, proteins and/or the amino acid histidine (making them unavailable as intracellular "building blocks") (Klasen, 2000; Hermans, 2006; Atiyeh *et al.*, 2007). Several methods have been used to prepare nAgs from silver ions: they are, for instances, chemical reduction, γ-ray irradiation and ultrasonication (Wang and Asher, 2001; Zhang *et al.*, 2001; Lu *et al.*, 2003). Recently, a variety of water-soluble polymers such as poly(vinyl alcohol) (PVA), poly(vinyl pyrolidone) (PVP), poly(ethylene glycol) (PEG) (Luo, 2005), gum acacia (Yu *et al.*, 2004; Dror *et al.*, 2006), cellulose-based polymers (Kwon *et al.*, 2005) and gelatin (Pal, 1997) have been used in the synthesis of nAgs, due mainly to their abilities to act as both the reducing agents and stabilizers.

Gelatin is a biopolymer derived from collagen via either acid or alkaline hydrolysis and, as such, it is biodegradable, biocompatible (Ward and Courts, 1997), non-toxic (Li *et al.*, 1998; Ugwoke and Kinget, 1998), naturally abundant and cheap. Its composition and biological properties are almost identical to its precursors. Gelatin has been widely used in pharmaceutical and medical fields as sealants for vascular prostheses, plasma expanders, ingredients in drug formulations, carriers for delivery of drugs or other therapeutic substances (Einerson *et al.*, 2003; Konishi *et al.*, 2003; Konishi *et al.*, 2005) and, especially, as wound-dressing materials (Tabata *et al.*, 1994; Tabata *et al.*, 1998; Lien *et al.*, 2008). However, gelatin, in general, exhibits a poor mechanical property that is too brittle when fully dried or too soft when fully wet and is easily soluble in aqueous media. To mitigate this, cross-linking can be introduced (Draye *et al.*, 1998; Mwangi and Ofner, 2004; Lien *et al.*, 2008).

Silk is easily available in Thailand. In general, *Bombyx mori* silk cocoon, from the cocoon of the domesticated mulberry silkworm, is composed of a fibrous

protein fibroin core (72-81 %) and a surrounding glue protein, sericin (19-28%) (Gamo *et al.*, 1977; Takasu *et al.*, 2002). Silk fibroin (SF) was widely used for biomedical applications, for example as an enzyme-immobilization material, as an oral dosage form (Hanawa, 1995), and as a burn wound dressing (Minoura *et al.*, 1990), SF has several useful properties including good biocompatibility (Wuand and Tian, 1996), good oxygen and water vapor permeability (Minoura *et al.*, 1990; Minoura *et al.*, 1990), minimal inflammatory reaction (Altman *et al.*, 2003) and slow degradation (Altman *et al.*, 2002). In addition, silk substrates have been shown to support human limbal epithelial stem cell attachment and proliferation on a comparable scale to tissue-culture plastic (Chirila *et al.*, 2008). Because of the excellent properties, SF was used as a biomaterial to make a wound dressing.

Hydrogels, due to their ability to imbibe large quantities of water and other aqueous media, are flexible, with properties that can be adjusted to resemble those of skin. Thus, their proposed uses have been in areas such as drug delivery devices, contact lens, cell transplantation matrices and wound dressings (Rathna *et al.*, 1996). As wound dressings, they should be able to maintain optimal amount of exudates that keeps the wound in a moist environment.

In the work, two types of natural biopolymers (i.e. gelatin and silk fibroin) were used to prepare the hydrogels. The nAg-loaded gelatin hydrogels and the nAg-loaded silk fibroin films were prepared by solution-casting technique. The $AgNO_3$-containing gelatin and silk fibroin solutions were aged under a mechanical stirring for various time intervals to allow for the formation of silver nanoparticles. The formation of nAgs was monitored by a UV-Vis spectrophotometer. The morphology and size of nAgs were characterized by transmission electron microscopy (TEM).

For gelatin, to improve the water resistance of the hydrogels, various contents of glutaraldehyde (GTA) were added to the $AgNO_3$-containing gelatin solution to cross-link the obtained gelatin hydrogels and the optimal concentration of GTA was studied by testing the water retention and weight loss behavior, release characteristic of the as-loaded silver, the antibacterial activity against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, and the indirect cytotoxic evaluation. Based on an indirect cytotoxicity evaluation, they showed toxicity towards normal human fibroblasts. The toxicity of the nAg-loaded gelatin

hydrogel pads was hypothesized to be a result of the presence of NO_3^- ions, which, upon ionic exchange with the less toxic metabisulfite anions, the hydrogels became less toxic to the fibroblastic cells (Rattanaruengsrikul *et al.*, 2009). The present contribution investigated further the effect of the initial concentration of $AgNO_3$ that would be loaded in the gelatin solution to finally obtain the nAg-loaded gelatin hydrogel pads. The major aim was to find an optimized concentration of $AgNO_3$ in the gelatin solution that resulted in the hydrogels with appropriated antibacterial activity against *E. coli* (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative) and *S. aureus* (Gram-positive) and less toxicity towards the fibroblastic cells.

For silk fibroin, the nAg-loaded silk fibroin films were treated with 90 vol.-% of methanol aqueous solution to improve the water resistance. These films were tested for their *in vitro* degradation behavior, release characteristic of the as-loaded silver by using an atomic absorption spectroscope (AAS), and antimicrobial activity for microorganisms.