CHAPTER VII
CONCLUSIONS AND RECOMMENDATION

7.1 Conclusions

In the present contribution, a gelatin solution containing silver nanoparticles (nAgs) was prepared from a gelatin solution containing silver nitrate ($AgNO_3$) in acetic acid or distilled water. The formation of nAg was achieved when the $AgNO_3$-containing gelatin solution was aged for various times under mechanical stirring. The formation of nAg was confirmed not only by the change in the color of the solution, but also by the observation of the surface plasmon peak in the UV spectrum at around 430-435 nm. The size of the nAgs that were formed in the $AgNO_3$-containing gelatin solution that had been aged for the proper time, as determined by TEM, ranged between 9-28 nm. The water retention and the loss in the weight of the cross-linked nAg-loaded gelatin hydrogel pads in three types of medium (i.e., acetate buffer, distilled water, and simulated body fluid buffer) were carried out to assess the effect of the glutaraldehyde (GTA) content used to cross-link the hydrogels. It was found that an increase in the GTA content resulted in the observed decrease in the values of both properties. Based on the water retention data in distilled water, the cross-link density of the hydrogels was determined and it was found to decrease with an increase in the GTA content used to cross-link the hydrogels. The total cumulative amount of silver released from the hydrogels was also found to decrease with an increase in the GTA content. The potential for use of the cross-linked nAg-loaded gelatin hydrogels as wound dressings was assessed by antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and indirect cytotoxicity against normal skin fibroblasts. All of the results showed that 1 μl/ml of GTA provided the optimal concentration for preparation the cross-linked nAg-loaded gelatin hydrogel as wound dressing because it provided high water retention, high total cumulative amount of silver released resulting in high antibacterial activity of the two pathogens and appeared to be less toxic against the tested cells only when the hydrogels were treated with a sodium metabisulfite solution.

The initial amount of $AgNO_3$ was further varied in 0.75, 1.0, 1.5, 2.0 and 2.5 wt.-% for preparing the nAg-loaded gelatin hydrogels. The concentrations of silver, either in the form of the free Ag^+ ions or the as-formed nAgs, within the obtained hydrogels that had been prepared from the gelatin solutions containing the initial $AgNO_3$ contents at 0.75, 1.0, 1.5, 2.0 and 2.5 wt.-% were determined to be 94.1, 124.5, 185.4, 247.1 and 307.3 $ppm \cdot g^{-1}$ of the hydrogels, respectively, on average. Within 24 h of submersion in the testing medium of either phosphate buffer saline solution (PBS) or simulated body fluid buffer (SBF), either about 40.5-56.4 % or 44.4-79.6 % of the as-loaded amounts of silver within the hydrogels was able to release into either medium, respectively. Antibacterial activity of these hydrogels was tested against Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and Gram-negative *Pseudomonas aeruginosa*, using the colony count method. After exposing the hydrogels to the microbial suspensions for 24 h, the numbers of the bacterial colonies were counted and it was found that the hydrogels, without or with the treatment with the sodium metabisulfite aqueous solution, could inhibit at least 99.77 % of the bacterial growth. Without the treatment with the sodium metabisulfite aqueous solution, the hydrogels, even at the lowest concentration of the as-loaded silver within the hydrogels (i.e., about 0.75 wt.-%), were detrimental to human's normal skin fibroblasts. Upon the treatment with the sodium metabisulfite aqueous solution, the viability of the cells was greatly improved. Direct culture of the cells onto the hydrogels containing about 0.75, 1.0 and 1.5 wt.-% of the as-loaded silver showed that the greater the amount of the as-loaded silver, the higher the toxicity. At about 1.5 wt.-% of the as-loaded silver, total detachment of the cultured cells was evident.

The nAg-loaded silk fibroin (SF) films were successfully prepared from a SF solution containing $AgNO_3$ with different concentrations in distilled water. The nAg was formed during aging these solutions at various times. The nAg could form in the SF solution due to the presence of SF acted as the reducing agent itself. The nAg in the SF solutions containing higher initial $AgNO_3$ contents were occurred faster than those containing lower initial $AgNO_3$ contents. The average size of the Ag particle that had been formed in the SF solutions containing 0.5, 1.0, 1.5, 2.0 and 2.5 wt.-% of $AgNO_3$ was about 7.72 ± 2.72, 8.74 ± 2.14, 8.98 ± 2.78, 9.86 ± 2.50 and

10.82 ± 3.25 nm, respectively. From *in vitro* degradation, the nAg loaded SF films could be proteolytically degraded with an increase time of exposure to the enzyme. The total cumulative amount of silver released from the films that had been prepared from the SF solutions containing higher initial $AgNO_3$ contents was greater than that containing lower initial $AgNO_3$ contents. The potential for use of the nAg-loaded SF films as wound dressings was assessed by antimicrobial activity against microorganism strains. The results showed that the films were effective against 6 strains such as *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *S. agalactiae*, *S. pyogenes* and *C. albicans*. Therefore, this study indicated that the nAg-loaded SF films could be possible used as antimicrobial wound dressings.

Table 7.1 Shows the comparative properties of between the nAg-loaded gelatin hydrogel and the nAg-loaded SF film

Properties	**The nAg-loaded gelatin hydrogel**		**The nAg-loaded silk fibroin film**
Antibacterial activity	Good	Good	Good
Mechanical property	Soft and flexible	Soft and flexible	Brittle
Water absorbability	Good	Good	Not good
Source	Easily available (mainly imported)	Easily available (mainly imported)	Easily available in Thailand
Solvent	Acetic acid	Distilled water	Distilled water
Preparation step/time:			
1.Raw material preparation	-	-	7 d
2.Solution preparation	5 d	8-15 h	1 – 1.5 d
3.Film preparation	6 d	6 d	1 d
The formation of nAg	5 d	8-15 h	24 - 36 h
Cost	~200-250 B.	~200-250 B.	~300 B.

7.2 Recommendation

The potential for use of the nAg-loaded gelatin hydrogels and the nAg-loaded SF films as antimicrobial wound dressings or skin regeneration applications should be further investigated *in vivo* or in an animal study.