CHAPTER VII
CONCLUSION AND RECOMMENDATIONS

7.1 Conclusions

In this research work, e-spun poly(acrylonitrile) (PAN; $M_w \approx 55{,}000$) and e-spun gelatin (GT; Bloom $\approx$ 180) fiber mats containing silver nanoparticles were successfully prepared by electrospinning process and developed for antibacterial membrane and wound dressing applications, respectively.

Ultrafine poly(acrylonitrile) (PAN) fiber mats were electrospun from a PAN solution (10%w/v) containing 0.5-2.5 wt% $AgNO_3$ under a fixed electric field of 15 kV/20 cm. Silver nanoparticles (nAg), a potent antibacterial agent, were generated by simple chemical reduction using N,N-dimethylformamide (DMF) as a solvent and reducing agent. The average diameters of the as-formed nAg were less than 5 nm. Electrospinning of both the base and the 5 d-aged $AgNO_3$-containing PAN solutions resulted in the formation of smooth fibers, with average diameters of ~650 and ~200 nm, respectively. The nAg-containing PAN fiber mats were further activated with UV irradiation to photoreduce residue Ag^+ and induced nAg migration to the surface of PAN. The releasing amount of nAg in distilled water was found to be increase after irradiation. The antibacterial activity of the ultrafine PAN fiber mats containing nAg against *S. Aureus* (Gram-positive), and *E. coli* (Gram-positive) was also more pronounced after UV irradiation.

Ultrafine gelatin fiber mats with antibacterial activity against some common bacteria found on burn wounds were prepared from a gelatin solution (22% w/v in 70 vol% acetic acid) containing 0.75, 1.00, 1.50, 2.00 and 2.5 wt% $AgNO_3$. Silver nanoparticles (nAg), a potent antibacterial agent, first appeared in the $AgNO_3$-containing gelatin solution after it had been aged for at least 12 h, with the amount of nAg increasing with increasing aging time. The average diameters of the as-formed nAg ranged between 10 and 20 nm. Electrospinning of both the base and the 12 h-aged 0.75-2.50 wt.% $AgNO_3$-containing gelatin solutions resulted in the formation of smooth fibers, with the average diameters being 260, 248, 226, 215 206 and 230 nm, respectively. The nAg-containing gelatin fiber mats were further cross-linked with moist glutaraldehyde vapor to improve their stability in an aqueous medium.

The release of Ag^+ ions from both the 1 h- and 3 h-cross-linked nAg (2.50 wt.% $AgNO_3$)-containing gelatin fiber mats – by the total immersion method in acetate buffer and distilled water (both at a skin temperature of 32°C) – occurred rapidly during the first 60 min, and increased gradually afterwards; while that in SBF (at the physiological temperature of 37°C) occurred more gradually over the testing period. Lastly, the antibacterial activity of these materials, regardless of the sample types, was greatest against *Pseudomonas aeroginosa*, followed by *Staphylococcus aureus*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus*, respectively. The release of Ag^+ ions from 0.5 h-cross-linked nAg (0.75-2.00 wt.% $AgNO_3$)-containing gelatin fiber mats, by the total immersion method in the acetate buffer and (at the skin temperature of 32°C) occurred rapidly during the first 60 min and increased gradually afterwards, while that in SBF (at the physiological temperature of 37°C) occurred less than that of in acetate buffer. Zone of inhibition of these materials, regardless of the sample types, was the greatest against *Bacillus subtilis*, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, respectively. For quantitative evaluation with antibacterial activity of the 0.75-2.00% $AgNO_3$-loaded e-spun GT fibers mats, it is found that the percentage of reduction reached 99 % with all of bacteria, indicating that the silver particles are responsible for the antibacterial activity of the $AgNO_3$-loaded electrospun gelatin fibers mats and this activity is quite strong.

The potential for use of the 0.75-2.00%-loaded e-spun GT fiber mats as wound dressings was assessed by investigating the cytotoxicity of these materials with normal human dermal fibroblast (NHDF) cells. The 0.75-1.00% $AgNO_3$-loaded e-spun GT fiber mats showed no threat towards normal human dermal fibroblasts. The in vitro and in vivo biocompatibility of e-spun fibrous membranes of gelatin and gelatin that contained 0.75-2.50% silver nitrate ($AgNO_3$) or silver nanoparticles (Agnano) were studied. In vitro human monocyte/macrophage cultures were used with the MTT test for cell viability and confocal microscopy to determine the adherent cell density on the respective materials at day 0 (2h), 3, 7 and 10. Statistical analyses were performed comparing the results of e-spun fibrous membranes of gelatin containing 0.75-2.50%$AgNO_3$ or Agnano at each time point with e-spun gelatin fibrous membranes. Only the 0.75%Agnano e-spun fibrous membranes were statistically comparable to the e-spun gelatin fibrous membranes. Based on the adherent cell density and cell viability data, all other $AgNO_3$ and Agnano were considered to be non-biocompatible or toxic. Statistical analysis demonstrated that the Agnano materials were less toxic than the $AgNO_3$ materials. For the in vivo study, samples of the materials were subcutaneously implanted in rats for 7, 14, and 28 days. After sacrifice, the tissue implant sites were histologically evaluated for inflammatory, foreign body reaction, and wound healing responses. Results from the in vivo studies were comparable to those from the in vitro studies with the e-spun gelatin fibrous membranes and the 0.75%Agnano demonstrating the earliest rapid resolution of the acute and chronic inflammatory responses with mild foreign body reactions and mild to moderate fibrous capsule formation at 14 and 28 days.

7.2 Recommendations

In this research work, only a single syringe was used in the electrospinning process, resulting in limited scale of production of electrospun fibers. In order to use this contribution in the industry, mass production of electrospun fibers is needed. Therefore, a multi-syringes electrospinning set-up should be used to achieve a large quantity of fibers within a shorter spinning time.

In case of the release study, it should be recommended that the modification of these electrospun fiber mats needed to be further studied. In order to develop this contribution, the antibacterial agent and the polymer matrix have to be appropriately chosen and/or modified to achieve the suitable release characteristic for specific applications.

In addition, the potential for use of silver-loaded polymer fiber mats as antibacterial dressing applications should be further investigated *in vivo* with infection wound.