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CHAPTER V

EFFECT OF CROSS-LINKING ON PROPERTIES AND RELEASE CHARACTERISTICS OF SODIUM SALICYLATE-LOADED ELECTROSPUN POLY(VINYL ALCOHOL) FIBER MATS

5.1 Abstract

Cross-linking of electrospun (e-spun) fiber mats of poly(vinyl alcohol) (PVA) containing sodium salicylate (SS), used as the model drug, was achieved by exposing the fiber mats to the vapor from 5.6 M aqueous solution of either glutaraldehyde or glyoxal for various exposure time intervals, followed by a heat treatment in a vacuum oven. With increasing the exposure time in the cross-linking chamber, the morphology of the e-spun fiber mats gradually changed from porous to dense structure. Both the degree of swelling and the percentage of weight loss of the cross-linked fiber mats (i.e., ~200-530% and ~15-57%, respectively) were lower than those of the untreated ones (i.e., ~610% and ~67%, respectively). Cross-linking was also responsible for the monotonous increase in the storage moduli of the cross-linked SS-loaded e-spun PVA fiber mats with increasing the exposure time in the cross-linking chamber. The release characteristic of the model drug from the SS-loaded e-spun PVA fiber mats both before and after cross-linking was assessed by the transdermal diffusion through a pig skin method. The cumulative release of the drug from these matrices could be divided into two stages: 0-4 and 4-72 h, in which the amount of SS released in the first stage increased very rapidly, while it was much slower in the second stage. Cross-linking slowed down the release of SS from the drug-loaded fiber mats appreciably and both the rate of release and the total amount of the drug released were decreasing functions of the exposure time interval in the cross-linking chamber. Lastly, the cross-linked SS-loaded e-spun PVA fiber mats were non-toxic to normal human dermal fibroblasts.

(**Key-words**: electrospinning; nanofibers; poly(vinyl alcohol); transdermal drug delivery; sodium salicylate)

5.2 Introduction

In recent years, electrospinning (e-spinning) process has received a great deal of attention due to its ability to produce ultrafine fibers with diameters in nanometer to sub-micrometer range and with high surface area to volume or mass ratios (Lu, 2001; Doshi, 1995). The principle of e-spinning process is the use of electrostatic force as the main driving force for fiber formation (Zhang, 2005; Shim, 2001; Theron, 2004). Morphology of the electrospun (e-spun) fibers depends on a number of parameters such as solution properties (e.g., concentration, viscosity, conductivity, surface tension, etc.), processing conditions (e.g., applied electrical potential, collection distance, collection time, etc.), and ambient conditions (e.g., temperature, humidity, etc.) (Shim, 2001; Ding, 2002). Among others, some potential uses for e-spun fibers in biomedical applications are, for examples, immobilization of enzyme (Wu, 2005), tissue-engineered scaffolds (Choi, 2004; Kim 2004), and delivery systems for DNA (Luu, 2003) and drugs (Kenawy, 2002; Zong, 2002; Zeng, 2003; Verreck 2003; Verreck 2003). One of the obvious advantages of the e-spinning process over the conventional solution-casting technique is the highly porous structure of the e-spun fiber mats in comparison with the dense structure of the as-cast films that offers greater surface area that would, assumingly, allow the drug molecules to diffuse out from the matrix much more conveniently (Kenawy, 2002; Zong, 2002)

The e-spun fibers from a good number of polymers have been developed as the matrix for the delivery of drugs. Poly(lactic acid) (PLA) and poly(ethylene-co-vinyl acetate) (PEVA) were successfully e-spun in the presence of tetracycline hydrochloride (an antibiotic drug) as a model drug by Kenawy et al. (Kenawy, 2002). The total percentage of tetracycline released from the as-cast films was lower than that from the e-spun fiber mats due to the much lower surface area. Zong et al. (Zong, 2003) also used PLA as the matrix and used mefoxin (an antibiotic drug) as the model drug. They arrived at a similar finding to that observed by Kenawy et al. (Kenawy, 2002). For poorly water-soluble drugs, such as itraconazole (an anti-fungal drug) and ketanserin (a drug for ischemic acute renal failure), polyurethane, a non-biodegradable polymer, was used as the matrix (Verreck, 2003). They

concluded that the release of poorly water-soluble drugs could be achieved using a water-insoluble polymer and the rate of release could be tailored by varying the drug to polymer ratio (Verreck, 2003).

Poly(vinyl alcohol) (PVA) is a hydrophilic, semi-crystalline polymer with good chemical and thermal stability. PVA is interesting here because of its biocompatibility, non-toxicity, good water permeability, and, particularly, excellent electro-spinnability (Zhang, 2005; Koski, 2004; Jun, 2005; Lee, 2004; Son, 2005; Yao, 2003; Chuangchote, 2006). Over the past few years, many researchers have investigated various parameters affecting morphology and diameters of e-spun PVA fibers: these parameters are, for examples, solution concentration, solution flow rate, applied electrical potential, collection distance, ionic salt addition, degree of hydrolysis of PVA (Zhang, 2005), molecular weight of PVA (Koski, 2004; Jun, 2005; Lee, 2004), pH (Son, 2005), surfactant addition (Yao, 2003), and type of collector (Chuangchote, 2006). Potential uses for the e-spun PVA fiber mats are, for examples, immobilization membranes for cellulase (Wu, 2005), delivery membranes for bovine serum albumin (BSA) (Zeng, 2005), and antimicrobial membranes from the e-spun PVA fiber mats that contained silver nanoparticles (Hong, 2006).

Recently, we reported successful preparation of e-spun PVA (degree of polymerization $\approx$ 1,600; degree of hydrolysis = 97.5-99.5%) fiber mats containing four different types of model drugs [e.g., sodium salicylate (SS), diclofenac sodium (DS), naproxen (NAP), and indomethacin (IND)] by e-spinning (applied electrical potential = 15 kV; collection distance = 15 cm; polarity of emitting electrode = positive; solution flow rate = 1 $ml \cdot h^{-1}$) (Taepaiboon, 2006). The release characteristics of the drugs from the e-spun fiber mats were compared with those of the drugs from the corresponding solution-cast films and the results indicated that the drug-loaded e-spun fiber mats showed much better release characteristics of the model drugs, both in terms of the total amount and the rate of release of the drugs, than the drug-loaded as-cast films, and the release mechanisms of the drugs from the drug-loaded e-spun fiber mats were mainly by the diffusion through the swollen PVA fibrous matrix and by the release due to partial dissolution of the matrix

(Taepaiboon, 2006). Control over the release characteristics of the drugs can be done through partial cross-linking of the PVA fibrous matrix.

In the present contribution, the e-spun fiber mats of PVA containing SS as the model drug were further cross-linked with the vapor from the aqueous solution of either glutaraldehyde (Chen, 2003; Praptowidodo, 2005) and glyoxal (Yang, 2005; Yang, 2005), followed by a heat treatment in an oven. It is believed that cross-linking by this method could minimize the toxicity of the cross-linked materials through the minimization of the unreacted or the incompletely-reacted chemical species (Ramires, 2002). Certain physical characteristics of the cross-linked SS-loaded e-spun PVA fiber mats and the release characteristic of SS from these fiber mats were investigated.

5.3 Experimental

5.3.1 Materials

Poly(vinyl alcohol) (PVA) (degree of polymerization ≈ 1,600 and degree of hydrolysis ≈ 97.5 to 99.5 mol.%) was purchased from Fluka (Switzerland). Sodium salicylate (SS) was purchased from Carlo Erba (Italy). This drug is used in the symptomatic management of painful and inflammatory conditions. Aqueous solutions of glutaraldehyde (5.6 M) and glyoxal (8.8 M) were purchased from Fluka (Switzerland). Sodium acetate (Ajax Chemicals, Australia) and glacial acetic acid (Carlo Erba, Italy) were of analytical reagent grade and used without further purification.

5.3.2 Preparation of SS-loaded electrospun PVA fiber mats

A weighed amount of PVA powder was dissolved in distilled water at 80°C for 3 h to prepare an aqueous PVA solution at a fixed concentration of 10% w/v. After the solution was cooled down to room temperature, the model drug was dissolved in the base PVA solution under constant stirring for 4 h prior to electrospinning (e-spinning). The drug was loaded at 20 wt.% based on the weight of the PVA powder.

E-spinning of both the base and the SS-containing PVA solutions was carried out by connecting the emitting electrode of positive polarity from a Gamma High-Voltage Research D-ES30PN/M692 high voltage DC power supply to the solutions contained in a standard 50-ml syringe, the open end of which was attached with a flat-tipped stainless steel gauge-20 needle (outside diameter = 0.91 mm), used as the nozzle, and the grounding electrode to a home-made rotating metal drum (width and outside diameter ≈ 15 cm), used as the fiber-collecting device. A fixed electrical potential of 15 kV was applied across a fixed collection distance of 15 cm (i.e., the electrostatic field strength of 15 kV/15 cm). The drum was rotated at a rotational velocity of about 50 to 65 rpm. The feed rate of the solutions was controlled at 1 $ml \cdot h^{-1}$ by means of a Kd Scientific syringe pump. For morphological study, the collection time was ~5 min, while, for the rest of the experiments, the collection time was ~24 h. After the 24-h spinning period, the thickness of the obtained fiber mats was in the range of 20 to 30 μm.

5.3.3 Cross-linking of SS-loaded electrospun PVA fiber mats

The SS-loaded electrospun (e-spun) PVA fiber mats were cross-linked by separate treatments with the vapor from an aqueous solution of either glutaraldehyde or glyoxal. The concentration of the solutions was fixed at 5.6 M (i.e., the concentration of the aqueous solution of glyoxal was diluted from its as-received aqueous solution). The treatments were carried out at a fixed temperature of 37°C for various exposure time intervals ranging from 0 to 8 h. After the treatments, the samples were further heated in a vacuum oven at 40°C for 30 h and, finally, kept in a desiccator for at least 5 d prior to further characterization.

5.3.4 Characterization of SS-loaded electrospun PVA fiber mats before and after cross-linking

Morphological appearance of SS-loaded e-spun PVA fiber mats both before and after cross-linking was observed by a JEOL JSM-5200 scanning electron microscope. The e-spun fiber mats were sputtered with a thin layer of gold prior to SEM observation. A Mettler Toledo 822e/400 differential scanning calorimeter (DSC) was used to investigate thermal behavior of the SS-loaded e-spun PVA fiber mats both before and after cross-linking. Each sample was subjected to a heating

scan over a temperature range of 25-350°C at a heating rate of 10°C min^{-1}. The measurement was carried out under nitrogen purge (60 ml min^{-1}).

A Rheometric Scientific ARES melt rheometer with a thin film/fiber fixture was used to study rheological properties of the SS-loaded e-spun PVA fiber mats both before and after cross-linking. In these experiments, the dynamic tension strain was applied and the storage moduli (E') were measured as a function of both strain (i.e., 0.01-10%) and frequency (i.e, 0.1-100 rad s^{-1}). The fiber mat samples were cut into a rectangular shape (6 mm x 70 mm; thickness = 20-30 μm) and placed on a thin film/fiber fixture sample holder (distance between two probes = 3 cm). Strain sweep tests were first carried out to determine the suitable strain(s) that allowed the E' measurements to be done in the linear viscoelastic regime (found to be 0.3% for all the fiber mat samples). Frequency sweep tests were then carried out to determine E' of each sample as a function of frequency. Each measurement was carried out at room temperature (~26 ± 1°C) and repeated at least two times.

Swelling and weight loss of the SS-loaded e-spun PVA fiber mats both before and after cross-linking were characterized in distilled water at 37°C for 24 h according to the following equations:

$$\text{Degree of swelling } (\%) = \frac{M - M_d}{M_d} \times 100, \tag{5.1}$$

and

$$\text{Weight loss } (\%) = \frac{M_i - M_d - M_r}{M_i - M_r} \times 100, \tag{5.2}$$

where M is the weight of each fiber mat sample after submersion in distilled water for 24 h, M_d is the weight of the sample in its dry state, M_i is the initial weight of the sample in its dry state, and M_r is the weight of the drug that was released from the sample.

5.3.5 Release of model drug from cross-linked SS-loaded electrospun PVA fiber mats

5.3.5.1 Preparation of acetate buffer

To prepare 1,000 ml of the acetate buffer solution, 150 g of sodium acetate was dissolved in 250 ml of distilled water. Exactly 15 ml of glacial acetic acid was then added very slowly into the aqueous sodium acetate solution.

Finally, distilled water was added into the solution to fill the volume. The pH of the as-prepared acetate buffer solution was 5.5.

5.3.5.2 Drug assay

The actual amount of the model drug in the SS-loaded e-spun PVA fiber mats both before and after cross-linking was quantified by dissolving or swelling the mats in 4 ml of dimethylsulfoxide (DMSO). After that, 0.5 ml of the solution was pipetted and added into 8 ml of the acetate buffer solution. Each drug-containing dilute solution sample was measured for the amount of the drug using a Perkin-Elmer Lambda 10 UV-spectrophotometer at a wavelength of 296 nm. The amount of the drug originally present in the SS-loaded e-spun PVA fiber mats both before and after cross-linking was back-calculated from the obtained data against a predetermined calibration curve of the drug. It should be noted that the presence of DMSO in the dilute solution had no obvious effect on the UV absorbance at the wavelength investigated. The results were reported as averages from at least 5 measurements.

5.3.5.3 Drug-release assay

The transdermal diffusion through a pig skin was carried out in order to study the release characteristic of the drug from SS-loaded e-spun PVA fiber mats both before and after cross-linking. Samples cut from the neat and the cross-linked SS-loaded as-spun PVA fiber mats were placed on a fresh piece of pig skin (abdomen; epidermal hair, subcutaneous fat, and underlying tissues removed; final thickness = 1 to 1.5 mm) which, in turn, was place on top of the acetate buffer solution on a modified Franz diffusion cell. At a specified diffusion period ranging between 0 and 72 h (3 d or 4,320 min), 0.3 ml of the buffer solution was withdrawn and an equal amount of fresh buffer solution was added into the cell to assure a good contact between the buffer solution and the skin at all times. The amount of the drug in the withdrawn solution samples was determined using the UV-spectrophotometer at the same wavelength previously mentioned against the predetermined calibration curve of the drug. The data were carefully calculated to determine the cumulative amount of the drug released from the samples at each specified diffusion period. The experiments were carried out in triplicate and the results were reported as average values.

5.3.6 Cytotoxicity evaluation

5.3.6.1 Cell culture

The target cells were normal human dermal fibroblasts (NHDF; 3rd passage). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

5.3.6.2 Indirect cytotoxicity assessment

This assay was carried out in adaptation from the ISO10993 and USP24 standard test methods. The cells were seeded in wells of a 96-well tissue-culture polystyrene plate (TCPS) at 2 000 cells/well and incubated for 48 h. Samples cut from the neat and the cross-linked SS-loaded e-spun PVA fiber mats were immersed in fresh culture medium for 24 h in incubation to produce extraction media of varying concentration (i.e., 1, 0.5, 0.125, and 0.031 $mg \cdot ml^{-1}$). Each of the as-prepared extraction media was then used to culture the cells and the cells were incubated for 24 h. The medium was then removed and the cells were further cultured with fresh culture medium and re-incubated further for another 24 h. The viability of the cells was then assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Plump, 1989) and the viability of the cells that were cultured with fresh culture medium was used as control.

5.3.6.3 Quantification of viable cells by MTT Assay

The MTT assay (Plump, 1989) is a tetrazolium dye-based colorimetric microtitration assay. Viable cells are able to metabolize tetrazolium (yellow) to formazan (purple) and the change in color can be measured spectrophotometrically. Briefly, 50 µl of MTT in phosphate buffer saline (PBS) (i.e., 5 $mg \cdot ml^{-1}$) was added to the medium in each cell-cultured well and the cells were incubated for 4 h. Medium and MTT were then aspirated from the wells and the formazan crystals were solubilized with 200 µl of dimethylsulfoxide (DMSO) and 25 µl of Sorenzen's glycine buffer (pH = 10.5). The optical density is read with a Victor 3 plate reader at a wavelength of 570 nm. The data were analyzed with SoftMax® Pro software (Molecular Devices, USA) to determine the inhibitory

concentration at 50% (IC_{50}) for all of the neat and the cross-linked drug-loaded e-spun PVA fiber mat samples investigated. Each data point was averaged from the measurements of 4 wells.

5.4 Results and discussion

5.4.1 Electrospinning of neat and SS-containing PVA solutions

The base PVA solution (i.e., 10% w/v in distilled water) was electrospun (e-spun) under an electrical potential of 15 kV over a collection distance of 15 cm (i.e., the electrostatic field strength of 15 kV/15 cm). The cross-sectionally round fibers with smooth surface were obtained with the average diameter being ~130 nm (see Figure 5.1a). Electrospinning (e-spinning) of the PVA solution containing 20 wt.% of sodium salicylate (SS) (based on the weight of PVA powder) was also successful with the same e-spinning condition as that of the base PVA solution (see Figure 5.1b). Clearly, beaded fibers were obtained. The dramatic increase in the conductivity of the PVA solution upon the addition of SS was postulated to be responsible for the bead-on-string morphology of the obtained fibers (Taepaiboon, 2006). Even though beads are usually seen as defects in the e-spinning process (Arayanarakul, 2006), whether smooth or beaded fibers were obtained is irrelevant here, as the present work only focused on the effect of cross-linking on the release characteristic of SS from both the neat and the cross-linked e-spun PVA fibrous matrix. Based on the SEM images observed for the e-spun fibers obtained from the SS-containing PVA solution, the average diameter of the fiber segments between beads was 107.8 ± 34.8 nm.

5.4.2 Cross-linking of SS-loaded electrospun PVA fiber mats

Since it was shown in our previous work (Taepaiboon, 2006) that the release mechanisms of the four model drugs, including SS, from the drug-loaded e-spun PVA fiber mats were mainly by the diffusion through the swollen PVA fibrous matrix and the release due to partial dissolution of the matrix, cross-linking of the e-spun PVA fibrous matrix should be carried out with an aim at controlling the release characteristics of the drugs from the matrix. Among the four model drugs studied in our previous work (Taepaiboon, 2006), SS showed the fastest and the greatest release

from both the e-spun fibrous and the as-cast film matrices, due possibly to the fact that SS is highly soluble in water and its molecular mass is the lowest among the drugs investigated. SS-loaded e-spun PVA fiber mats were then chosen as the model system for further investigation of the effect of cross-linking on their release characteristic of the drug.

Cross-linking of the SS-loaded e-spun PVA fiber mats was carried out by exposing the mats in a chamber which was saturated with the vapor of glutaraldehyde or glyoxal from their corresponding aqueous solution (i.e., 5.6 M). The exposure time was varied from 30 min to 8 h, depending on the type of the cross-linking agent. After the treatments, the samples were further heated in a vacuum oven at 40°C for a period of 30 h and kept in a desiccator for at least 5 d prior to further characterization. The use of the vapor in cross-linking the as-spun PVA fiber mats is to prevent the collapse (i.e., partial or complete dissolution) of the porous structure into a dense membrane during cross-linking in an aqueous phase (Rho, 2006). The cross-linking reaction of PVA with glutaraldehyde was reported elsewhere (Praptowidodo, 2005).

Table 5.1 shows selected SEM images of the SS-loaded e-spun PVA fiber mats after cross-linking treatments with the vapor from the aqueous solution of either glutaraldehyde or glyoxal at various exposure time intervals. Evidently, increasing the exposure time in the chamber caused adjacent fiber segments to fuse to one another, a result of the partial dissolution of the fiber segments when they came into contact with the moisture-rich vapor from the aqueous solution of either glutaraldehyde or glyoxal. Interestingly, at the same exposure time intervals, the SS-loaded e-spun PVA fiber mats which were exposed to the vapor from the aqueous solution of glutaraldehyde exhibited more interconnection of the adjacent fiber segments than those exposed to that of glyoxal.

Since the vapor pressure of glyoxal (i.e., ~18 mmHg @ 20°C for 40% aqueous solution) is slightly greater than that of water (i.e., ~17.5 mmHg @ 20°C), while it is much greater than that of glutaraldehyde (i.e., 15 mmHg @ 20°C for 50% aqueous solution), the vapor from the aqueous solution of glutaraldehyde used to treat the SS-loaded e-spun PVA fiber mats in this work would contain a greater

amount of water molecules than that from the corresponding solution of glyoxal. This would cause the adjacent fiber segments of the treated SS-loaded e-spun PVA fiber mats to fuse to one another at a greater extent when compared with the mats that were treated with the vapor from the aqueous solution of glyoxal. The change in the morphology from fibrous to film-like structure of the fiber mats that were treated with the vapor from the aqueous solution of glutaraldehyde occurred at ~5 h, while that of the fiber mats that were treated with the vapor from the aqueous solution of glyoxal did so at ~8 h.

5.4.3 Characterization of cross-linked SS-loaded electrospun PVA fiber mats

5.4.3.1 Swelling and weight loss behavior

Swelling and weight loss of the SS-loaded e-spun PVA fiber mats both before and after cross-linking treatments with the vapor from the aqueous solution of either glutaraldehyde or glyoxal at various exposure time intervals are summarized in Table 5.2. The weight of the fiber mat samples after submersion in distilled water at 37°C for 24 h both in their wet and dry states was recorded to arrive at the degree of swelling and the percentage of weight loss shown in the Table. Without cross-linking, the degree of swelling of the neat e-spun PVA fiber mat was ~610%, while the percentage of weight loss was as much as ~67%. After cross-linking treatments, both the degree of swelling and the percentage of weight loss for the SS-loaded e-spun PVA fiber mats showed a monotonous decrease in both values with increasing the exposure time in the cross-linking chamber.

Specifically, for the fiber mats treated with the vapor from the aqueous solution of glutaraldehyde, the degree of swelling decreased from that of the untreated fiber mat (i.e, ~610%) to ~530% at the exposure time of 0.5 h and finally to ~220% at the exposure time of 5 h, while the percentage of weight loss decreased from that of the untreated fiber mat (i.e., ~67%) to ~57% at the exposure time of 0.5 h and finally to ~16% at the exposure time of 5 h. For those treated with the vapor from the aqueous solution of glyoxal, the degree of swelling decreased from that of the untreated fiber mat (i.e, ~610%) to ~380% at the exposure time of 1 h and finally to ~200% at the exposure time of 8 h, while the percentage of weight loss decreased

from that of the untreated fiber mat (i.e., ~67%) to ~29% at the exposure time of 1 h and finally to ~15% at the exposure time of 8 h.

The monotonous decrease in both the degree of swelling and the percentage of weight loss of the fiber mats after cross-linking treatments could be due to the cross-linking reactions between the hydroxyl groups of PVA and the aldehyde groups of the cross-linking agents (Praptowidodo, 2005) and to the decrease in the total surface area of the fiber mats as the mats changed from the porous into the dense structure when they came into contact with the moisture in the treating vapor (Wu, 2005). According to Table 5.2, the fact that the fiber mats that were treated with the vapor from the aqueous solution of glutaraldehyde exhibited the lower values of both the swelling and the weight loss than those of the mats that were treated with the vapor from the solution of glyoxal indicated that glutaraldehyde was the more efficient cross-linking agent for PVA.

5.4.3.2 Thermal properties

Previously, we reported that the neat e-spun PVA fiber mat exhibited a loss of moisture coupled with a glass transition over a temperature range of ~40 to 120°C, a melting peak temperature (T_m) of ~230°C, and a thermal degradation peak temperature (T_d) of ~305°C (Taepaiboon, 2006). We also reported that the neat SS-loaded e-spun PVA fiber mat showed a loss of moisture coupled with a glass transition over a similar temperature range, while it exhibited much lower T_m and T_d values (i.e., ~200 and 255°C, respectively) than those of the neat e-spun PVA fiber mat (Taepaiboon, 2006). Figure 5.2a shows DSC thermograms for the neat SS-loaded e-spun PVA fiber mat and the mats that were treated with the vapor from the aqueous solution of glutaraldehyde at various exposure time intervals. The obtained data for the mats that were treated with the vapor from the aqueous solution of glyoxal are shown in Figure 5.2b. Evidently, both the neat and the cross-linked SS-loaded e-spun PVA fiber mat samples exhibited similar T_m and T_d values to those of the neat SS-loaded e-spun PVA fiber mat sample previously reported (Taepaiboon, 2006). Specifically, these samples exhibited the T_m values in the range of ~200 to 205°C and the T_d values in the range of ~255 to 260°C.

A careful consideration of the low-temperature endotherm (over the temperature range of ~40 to 120°C, corresponding to the loss of moisture coupled with a glass transition, revealed a shift in its peak value towards a higher temperature with increasing the exposure time in the cross-linking chamber. Since, according to a separate thermogravimetric analysis, the moisture content of both the neat and the cross-linked SS-loaded e-spun PVA fiber mats varied only in a small range of ~6 to 8% (results not shown), the shift in the peak values of the low-temperature endotherm for these samples should correspond to the change in the glass transition behavior of the fiber mats after cross-linking treatments. The steady, but slight, increase in the glass transition temperature of the fiber mats after cross-linking treatments is an indication of the decrease in the mobility of the PVA molecules upon cross-linking with either glutaraldehyde or glyoxal molecules (Park, 2001).

5.4.3.3 Rheological properties

The storage moduli (E') under a strain sweep test for both the neat and the cross-linked SS-loaded e-spun PVA fiber mats that were treated with the vapor from the aqueous solution of glyoxal are shown in Figure 5.3b. The obtained data for the mats that were treated with the vapor from the aqueous solution of glutaraldehyde are shown in Figure 5.3a. Evidently, the neat SS-loaded e-spun PVA fiber mat exhibited a constancy in the E' values over a small strain region in the range of 0.01 to ~0.9%, after which point E' values decreased rather monotonically with further increase in the strain. The critical strain of ~0.9% was determined from the intersection of the tangent lines. A similar behavior was also observed on all of the cross-linked SS-loaded e-spun PVA fiber mats, with a slight increase in the critical strain that the E' values started to deviate from the plateau region. At any given strain, the E' values of the cross-linked SS-loaded e-spun PVA fiber mats were much greater than those of the neat one, and, with increasing the exposure time in the cross-linking chamber, E' showed a monotonous increase in its values. Interestingly, the SS-loaded e-spun PVA fiber mats that were cross-linked with glyoxal vapor exhibited much greater E' values in comparison with those cross-linked with glutaraldehyde vapor. A similar result was observed on the cross-linked xerogels of poly(acrylic acid) (PAA) and poly(vinyl alcohol-co-vinyl acetate)

(PVAA) blends, in which the glyoxal-cross-linked xerogels exhibited a modulus almost twice as high as glutaraldehyde-cross-linked ones (Cauich-Rodriguez, 1996).

The storage moduli (E') under a frequency sweep test for both the neat and the cross-linked SS-loaded e-spun PVA fiber mats that were treated with the vapor from the aqueous solution of glutaraldehyde are shown in Figure 5.4a. The obtained data for the mats that were treated with the vapor from the aqueous solution of glyoxal are shown in Figure 5.4b. Apparently, the neat SS-loaded e-spun PVA fiber mat exhibited a slight, monotonous increase in the E' values as the frequency increased from 0.1 to 100 rad·s^{-1}. As the frequency increased from 0.1 to 100 rad·s^{-1}, the observational time decreased significantly, causing a polymeric material to appear stiffer. A similar behavior was also observed on all of the cross-linked SS-loaded e-spun PVA fiber mats. At any given frequency, the E' values of the cross-linked SS-loaded e-spun PVA fiber mats were much greater than those of the neat one, and, with increasing the exposure time in the cross-linking chamber, E' exhibited a monotonous increase in its values. Again, the SS-loaded e-spun PVA fiber mats that were cross-linked with glyoxal vapor exhibited much greater E' values in comparison with those cross-linked with glutaraldehyde vapor.

5.4.4 Release characteristic of SS from cross-linked SS-loaded electrospun PVA fiber mats

Prior to investigating the release characteristic of the model drug from both the neat and the cross-linked SS-loaded e-spun PVA fiber mats, the actual amount of the drug within these samples needed to be determined. Previously, we reported that the actual amount of SS within the e-spun fiber mat from the PVA solution that contained the initial amount of SS at 20 wt.% was ~81 ± 1% (Taepaiboon, 2006). In the present work, a slightly greater value (i.e., ~88 ± 1%) was observed on all types of samples. This value was further used to arrive at the cumulative release of the drug from both the neat and the cross-linked SS-loaded e-spun PVA fiber mats.

In the present work, the release characteristic of the drug from all types of the samples was carried out by the transdermal diffusion through a pig skin method. The experiments were carried out using acetate buffer as the transferring

medium at the physiological temperature of 37°C. Figure 5.5 shows the cumulative release of SS from the neat SS-loaded e-spun PVA fiber mat and the corresponding fiber mats that were cross-linked with the vapor from the aqueous solution of either glutaraldehyde or glyoxal at various exposure time intervals. The amount of SS released from the neat SS-loaded e-spun PVA fiber mat increased very rapidly over the first 4 h, after which time it increased gradually with further increase in the diffusion time. Obviously, cross-linking slowed down the release of SS from the drug-loaded fiber mats appreciably and the rate of release was qualitatively a decreasing function of the exposure time in the cross-linking chamber, a direct result of the decrease in both the degree of swelling and the surface area of the cross-linked fiber mats with increasing the exposure time (see Tables 5.1 and 5.2).

In addition to the qualitative decrease in the rate of release of the drug from the cross-linked SS-loaded e-spun PVA fiber mats, the total amount of the drug released from the SS-loaded fiber mats was also affected by the cross-linking treatments. Based on the results shown in Figure 5.5, the total amount of the drug released from the glutaraldehyde-cross-linked SS-loaded e-spun PVA fiber mats (i.e., at 72 h) was found to decrease from that of the neat fiber mat (i.e., 75%) to ~73, 67, 55, and 46% for the cross-linking exposure times of 0.5, 1, 3, and 5 h, respectively, while the that of the drug released from the glyoxal-cross-linked fiber mats was found to decrease from that of the neat fiber mat (i.e., 75%) to ~68, 65, 66, and 53% for the cross-linking exposure times of 1, 3, 5, and 8 h, respectively. At the same exposure times, the total amount of the drug released from the SS-loaded e-spun PVA fiber mats that were cross-linked with the vapor from the aqueous solution of glutaraldehyde was consistently lower than that of the drug released from the corresponding fiber mats that were cross-linked with the vapor from the solution of glyoxal, a direct result of the lower values of both the swelling and the weight loss of the glutaraldehyde-cross-linked fiber mats in comparison with those of the glyoxal-cross-linked counterparts.

5.4.5 Release kinetics of SS from cross-linked SS-loaded electrospun PVA fiber mats

The release kinetics of a drug from a carrier is often characterized using an equation of the following form (Ritger, 1987; Peppas, 1993):

$$\frac{M_t}{M_\infty} = kt^n, \text{ for } \frac{M_t}{M_\infty} < 0.6, \qquad (5.3)$$

where M_t is the cumulative amount of the drug released at an arbitrary time t, M_∞ is the cumulative amount of the drug released at an infinite time, n is an exponent characterizing the mechanism with which the release kinetics can be described, and k is the rate of release of the drug that incorporates physical characteristics of the matrix/drug system as well as some physical contributions from the measurement methods (viz. in the case of the transdermal diffusion through a pig skin method which involves the diffusion of the drug through a pig skin).

The analyses for the release kinetics of SS from both the neat and the cross-linked SS-loaded e-spun PVA fiber mats for the data shown in Figure 5.5 based on the Fickian diffusion type of the release mechanism (i.e., n = 0.5) were carried out in two stages. The first stage of the release was during the first 4 h of the diffusion period (hereafter, the rate parameter k associated with this period denotes k_1), while the second stage was during 4-72 h period (hereafter, the rate parameter k associated with this period denotes k_2). The biphasic release was also observed and reported by others (Verreck, 2003). The results from such analyses are summarized in Table 5.3.

For the drug that was release from the neat SS-loaded e-spun PVA fiber mat, the rate parameter k for the first and the second stages of the release (i.e., k_1 and k_2, respectively) were 0.028 and 0.007 $s^{-0.5}$, respectively. Evidently, cross-linking of the drug-loaded fiber mats caused the k_1 values to decrease monotonically with increasing the exposure time in the cross-linking chamber, while it did not affect much the k_2 values. Emphatically, at the same exposure times, the k_1 values of the drug released from the SS-loaded e-spun PVA fiber mats that were cross-linked with the vapor from the aqueous solution of glutaraldehyde was consistently lower than those of the drug released from the corresponding fiber mats that were cross-linked with the vapor from the solution of glyoxal, a result that complimented with the results on the total amount of the drug released from these matrices.

5.4.6 Cytotoxicity evaluation

The cytotoxicity of both the neat and the cross-linked SS-loaded e-spun PVA fiber mats was evaluated indirectly from the extraction media prepared by

immersing the fiber mat samples in fresh culture medium for 24 h in incubation. Four concentrations of the extraction media (i.e., 1, 0.5, 0.125, and 0.031 $mg \cdot ml^{-1}$) were prepared for each sample type. Each of the extraction media was then used to culture normal human dermal fibroblasts (NHDF) for 24 h.

Table 5.4 summarizes the number of viable cells (reported as the percentage of the initial number of seeded cells of 2 000 cells/well) after the cells were culture with the extraction media. Apparently, the percentage of viable cells for all of the fiber mat samples was greater than or equal to 75%. At the same exposure times, the percentage of viable cells for the SS-loaded e-spun PVA fiber mats that were cross-linked with the vapor from the aqueous solution of glyoxal was comparatively greater than that for the fiber mats that were cross-linked with the vapor from the solution of glutaraldehyde, a strong indication of the less toxicity of the materials towards the skin cells. Obviously, the inhibitory concentration at 50% (IC_{50}) for these materials was more than 1 $mg \cdot ml^{-1}$. Interestingly, despite the well-known toxicity of glutaraldehyde (Scobbie, 1995), its toxicity was minimized by the cross-linking procedure utilized in the present work. In addition, the morphology of NHDF that were cultured on the surfaces of the neat and some of the cross-linked SS-loaded e-spun PVA fiber mats assumed their normal appearance and spreaded well over the surfaces of the fiber mats (see Figure 5.6).

5.5 Conclusions

Cross-linking of electrospun (e-spun) fiber mats (beaded fiber morphology with the average diameter of the fiber segments between beads being ~108 nm) of poly(vinyl alcohol) (PVA) containing sodium salicylate (SS) (20 wt.% based on the weight of PVA powder), used as the model drug, was achieved by exposing the fiber mats to the vapor from 5.6 M aqueous solution of either glutaraldehyde or glyoxal in a closed chamber for various exposure time intervals ranging from 0 to 8 h at a constant temperature of 37°C, followed by a heat treatment in a vacuum oven at 40°C for 30 h. With increasing the exposure time in the cross-linking chamber, the morphology of the e-spun fiber mats gradually changed from porous to dense

structure. Swelling and weight loss of the SS-loaded e-spun PVA fiber mats both before and after cross-linking treatments were determined by submersion of the fiber mat samples in distilled water at 37°C for 24 h. Both the degree of swelling and the percentage of weight loss of the cross-linked fiber mats (i.e., ~200-530% and ~15-57%, respectively) were lower than those of the untreated fiber mats (i.e., ~610% and ~67%, respectively). Cross-linking was also responsible for the monotonous increase in the storage moduli of the cross-linked SS-loaded e-spun PVA fiber mats with increasing the exposure time interval in the cross-linking chamber. Although glutaraldehyde was shown to be the more effective cross-linking agent for PVA than glyoxal, as suggested by the swelling and weight loss results, glyoxal-cross-linked SS-loaded e-spun PVA fiber mats appeared to be much stronger than the glutaraldehyde-cross-linked counterparts.

The release characteristic of the model drug from the SS-loaded e-spun PVA fiber mats both before and after cross-linking was assessed by the transdermal diffusion through a pig skin method, using acetate buffer as the transferring medium, at 37°C. The cumulative release of the drug from these samples could be divided into two stages: 0-4 and 4-72 h, in which the amount of SS released in the first stage increased very rapidly, while it was much slower in the second stage. Evidently, cross-linking slowed down the release of SS from the drug-loaded fiber mats appreciably and both the rate of release and the total amount of the drug released were decreasing functions of the exposure time interval in the cross-linking chamber. In line with the observed lower values of both the swelling and the weight loss of glutaraldehyde-cross-linked fiber mats in comparison with those of the glyoxal-cross-linked counterparts, both the rate (in the first stage) and the total amount of the drug released from glutaraldehyde-cross-linked fiber mats was consistently lower than those of the drug released from the the glyoxal-cross-linked counterparts. Lastly, the cross-linking procedure used in this work rendered the corresponding cross-linked SS-loaded e-spun PVA fiber mats to be non-toxic to normal human dermal fibroblasts.

5.6 Acknowledgments

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Table 5.1 Selected scanning electron micrographs (magnification = 10,000x; scale bar = 1 μm) of sodium salicylate-loaded electrospun PVA fiber mats after cross-linking with the vapor from aqueous solution of either glutaraldehyde or glyoxal at various exposure time intervals

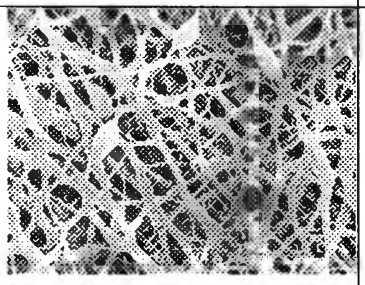
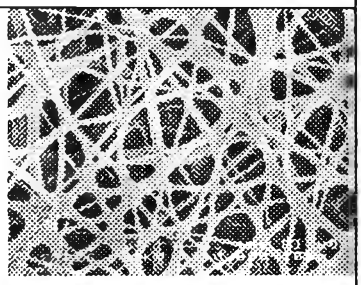
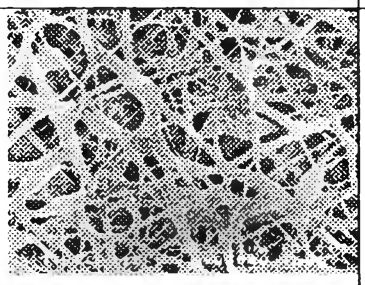
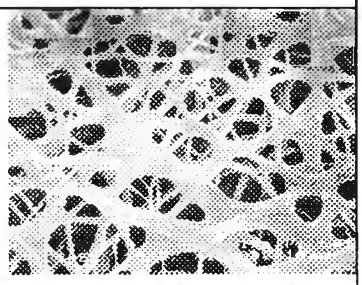
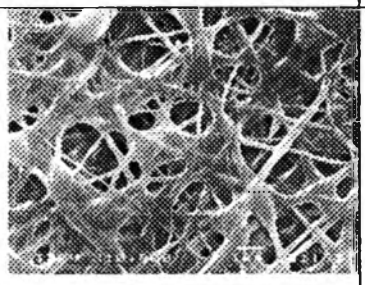
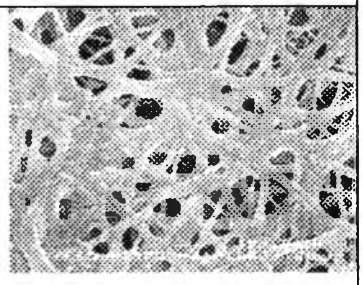
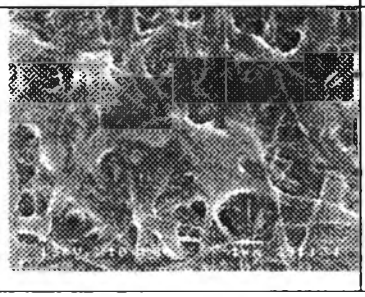
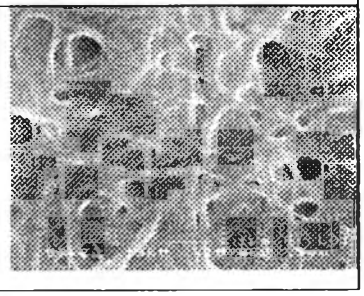
Exposure time (h)	Cross-linked with glutaraldehyde vapor	Exposure time (h)	Cross-linked with glyoxal vapor
0.5		1	
1		3	
3		5	
5		8	

Table 5.2 Degree of swelling (%) and weight loss (%) of sodium salicylate-loaded electrospun PVA fiber mats before and after cross-linking with the vapor from aqueous solution of either glutaraldehyde or glyoxal at various exposure time intervals

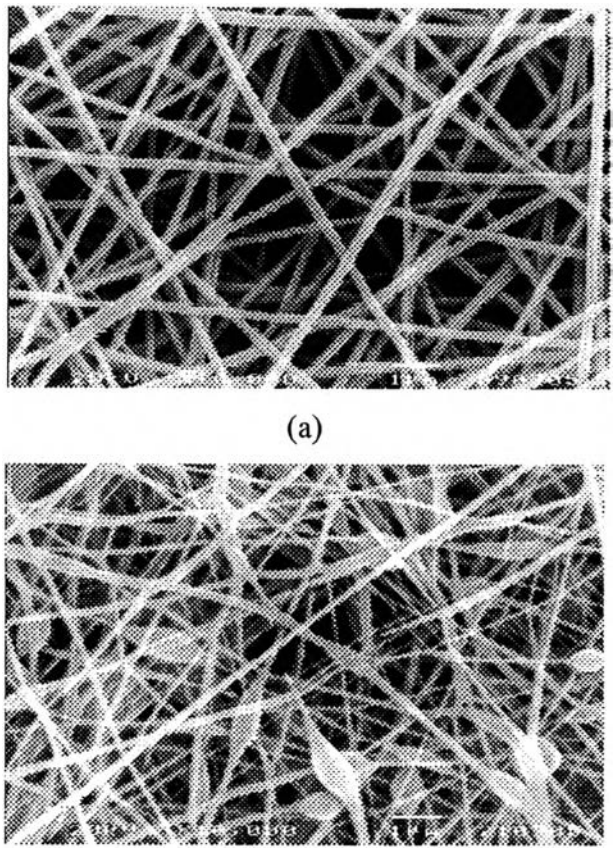
Sample type	Exposure time (h)	Degree of swelling (%)	Weight loss (%)
Neat	-	610 ± 29	66.5 ± 2.5
Cross-linked with glutaraldehyde vapor	0.5	529 ± 19	56.6 ± 2.8
	1	366 ± 14	27.0 ± 2.0
	3	293 ± 13	22.4 ± 1.4
	5	215 ± 17	16.3 ± 4.6
Cross-linked with glyoxal vapor	1	377 ± 11	29.2 ± 1.8
	3	300 ± 12	25.4 ± 2.0
	5	224 ± 11	21.4 ± 1.8
	8	203 ± 12	14.5 ± 1.5

Table 5.3 Analyses of the release kinetics of sodium salicylate from neat and cross-linked sodium salicylate-loaded electrospun PVA fiber mats based on the Fickian diffusion type of release mechanism

Sample type	Exposure time (h)	0-4 h		4-72 h	
		Rate parameter k ($s^{-0.5}$)	r^2	Rate parameter k ($s^{-0.5}$)	r^2
Neat	-	0.028	1.00	0.007	0.98
Cross-linked with glutaraldehyde vapor	0.5	0.019	0.98	0.013	1.00
	1	0.017	1.00	0.009	0.99
	3	0.015	0.99	0.008	1.00
	5	0.008	0.98	0.007	0.97
Cross-linked with glyoxal vapor	1	0.023	0.98	0.007	1.00
	3	0.021	0.99	0.008	0.99
	5	0.017	1.00	0.009	0.99
	8	0.013	1.00	0.008	0.99

Table 5.4 Indirect cytotoxicity evaluation of sodium salicylate-loaded electrospun PVA fiber mats before and after cross-linking with the vapor from aqueous solution of either glutaraldehyde or glyoxal at various exposure time intervals

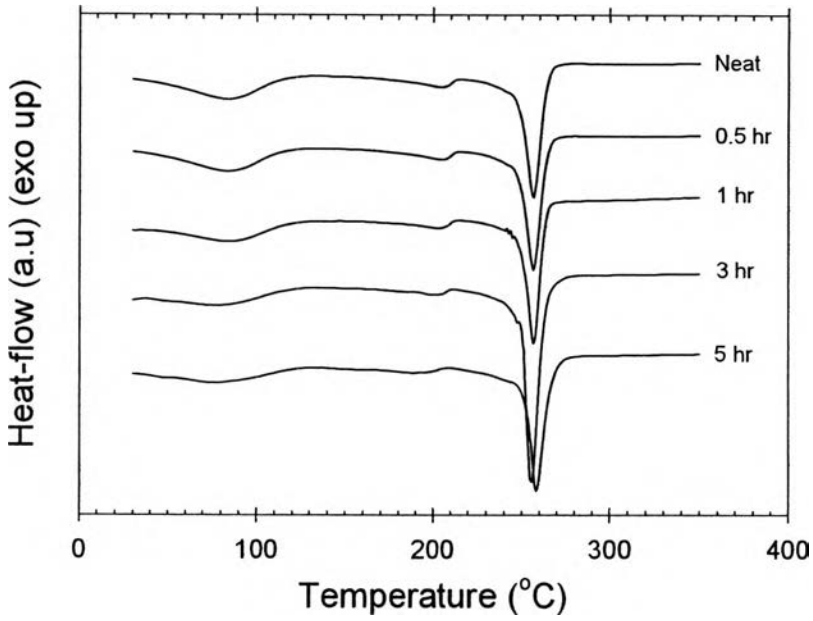
Sample type	**Exposure time (h)**	**Percentage of survived normal human dermal fibroblasts (NHDF) after being cultured with the extraction medium at each specified concentration ($mg \cdot ml^{-1}$)**				**IC_{50} ($mg \cdot ml^{-1}$)**
		1	**0.5**	**0.125**	**0.031**	
Neat	-	88 ± 0	90 ± 3	92 ± 2	91 ± 2	>1
Cross-linked with glutaraldehyde vapor	0.5	75 ± 2	81 ± 3	79 ± 0	84 ± 3	>1
	1	85 ± 1	86 ± 2	94 ± 3	92 ± 0	>1
	3	83 ± 1	88 ± 2	86 ± 2	94 ± 1	>1
	5	75 ± 2	82 ± 1	81 ± 0	87 ± 4	>1
Cross-linked with glyoxal vapor	1	100 ± 12	> 100	> 100	94 ± 5	>1
	3	90 ± 7	90 ± 5	92 ± 3	89 ± 7	>1
	5	> 100	> 100	> 100	> 100	>1
	8	93 ± 2	100 ± 2	95 ± 1	94 ± 2	>1



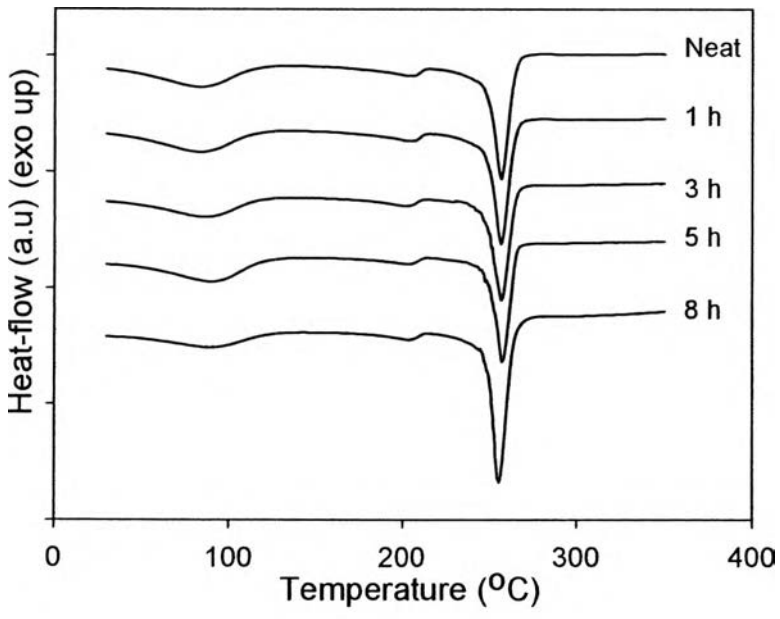
(a)

(b)

Figure 5.1 Selected scanning electron micrographs (magnification = 10,000x; scale bar = 1 μm) of a) electrospun fiber mats from 10% w/v PVA solutions and b) electrospun fiber mats from 10% w/v PVA solutions with the addition of 20% sodium salicylate (based on the weight of PVA powder). The electrostatic field strength was 15 kV/15 cm and the collection time was 5 min.



(a)



(b)

Figure 5.2 Differential scanning calorimetric thermograms of neat sodium salicylate-loaded electrospun PVA fiber mat (denotes as "neat") and the mats that were cross-linked with the vapor from the aqueous solution of (a) glutaraldehyde and (b) glyoxal at various exposure time intervals.

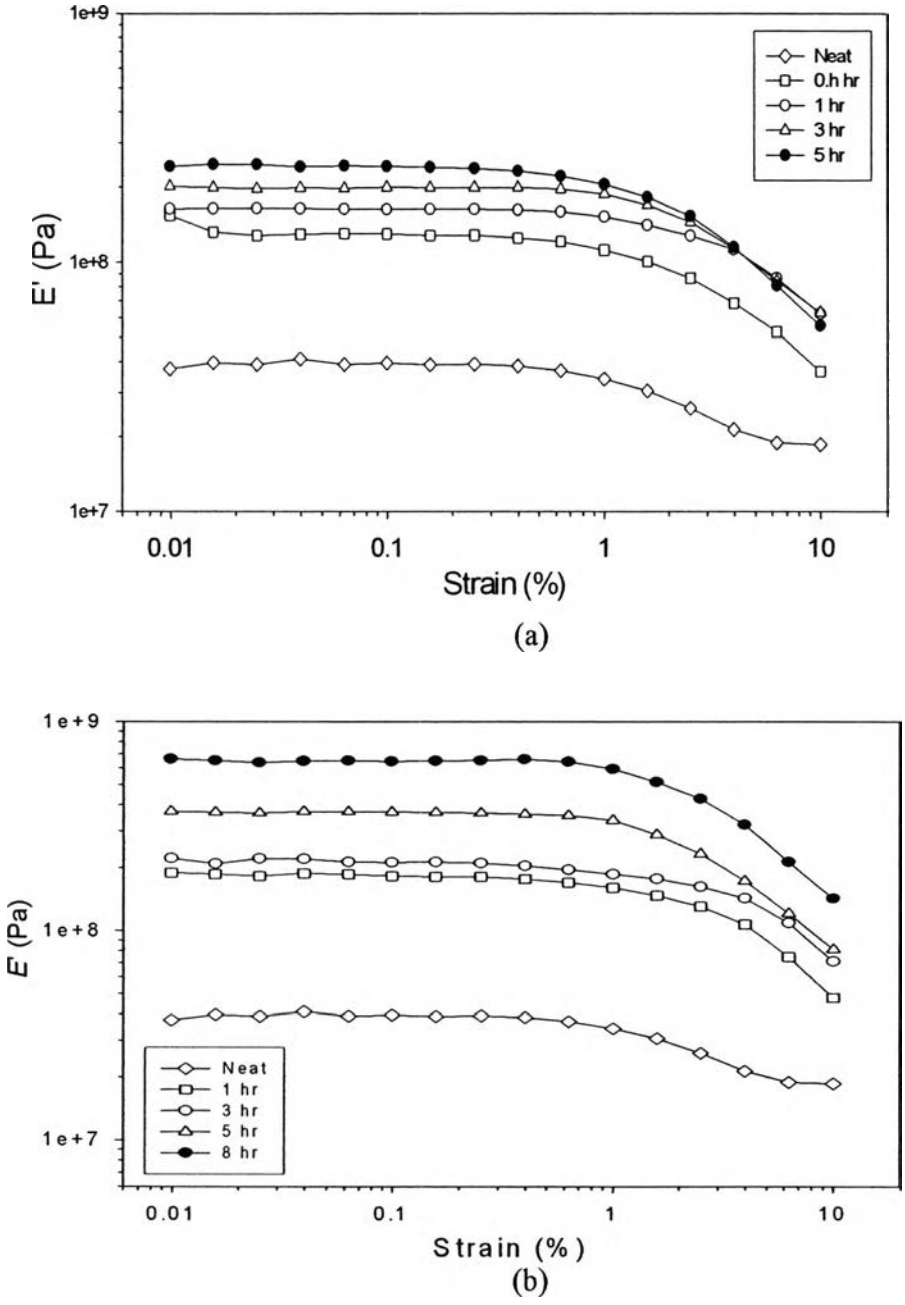
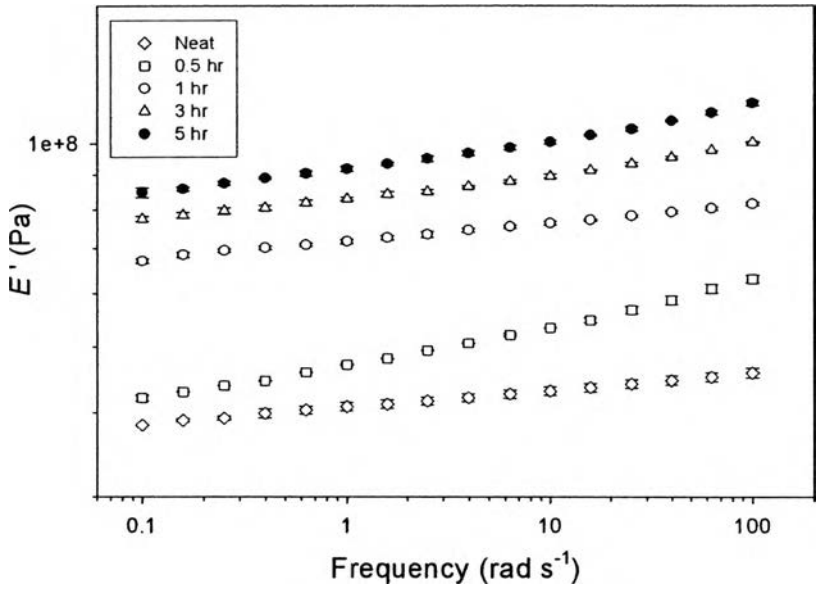
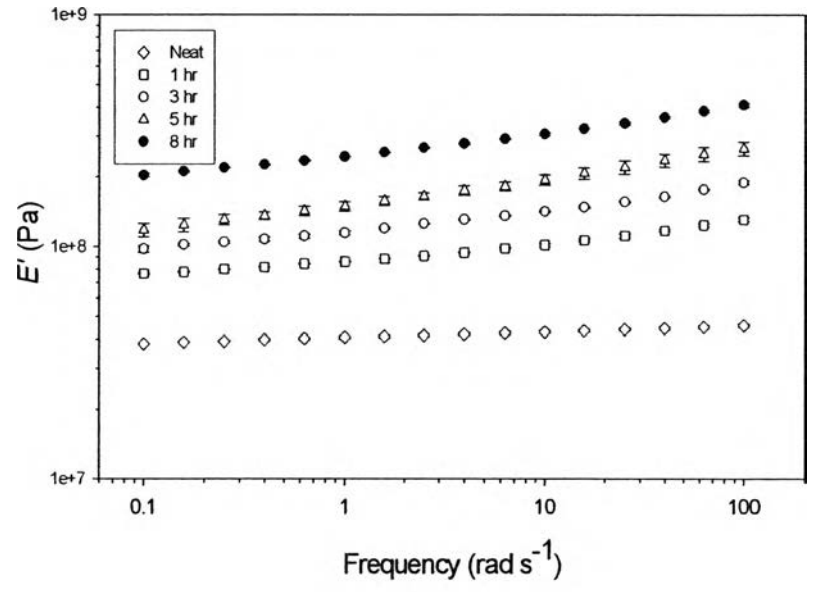


Figure 5.3 Strain sweep test results illustrating the storage moduli (E') as a function of strain (0.01-10%) at a fixed frequency of 1 rad·s^{-1} of neat sodium salicylate-loaded electrospun PVA fiber mat (denotes as "neat") and the mats that were cross-linked with the vapor from the aqueous solution of (a) glutaraldehyde and (b) glyoxal at various exposure time intervals. The test temperature was 26 ± 1°C.

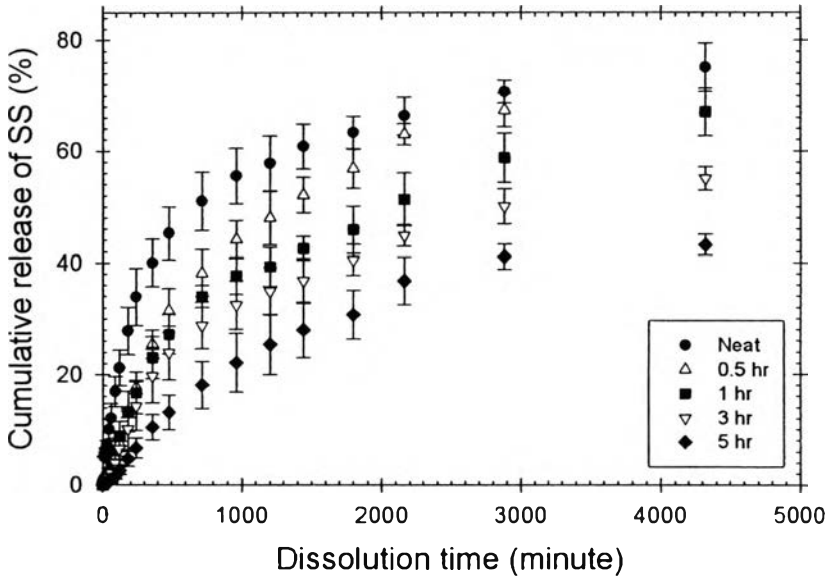


(a)

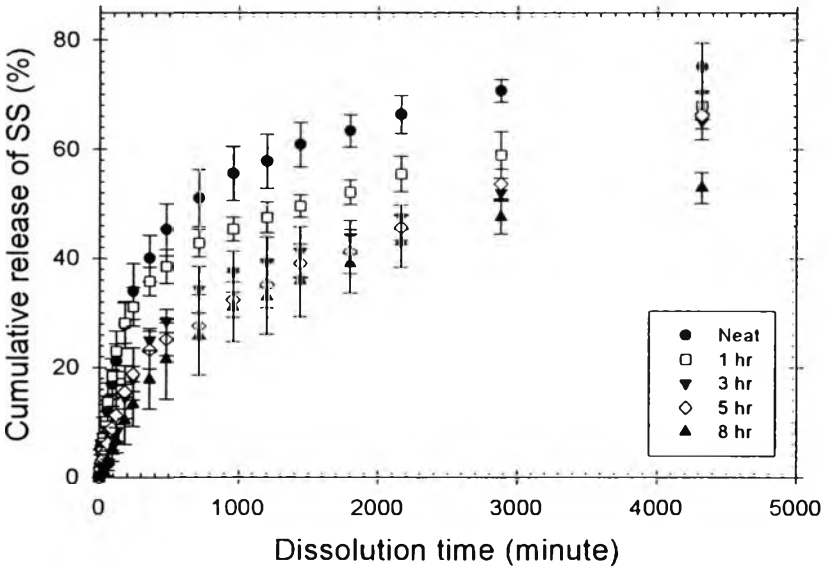


(b)

Figure 5.4 Frequency sweep test results illustrating the storage moduli (E') as a function of frequency (0.1-100 rad·s^{-1}) at a fixed strain of 0.5% of neat sodium salicylate-loaded electrospun PVA fiber mat (denotes as "neat") and the mats that were cross-linked with the vapor from the aqueous solution of (a) glutaraldehyde and (b) glyoxal at various exposure time intervals. The test temperature was 26 ± 1°C.



(a)



(b)

Figure 5.5 Profile of sodium salicylate released from neat sodium salicylate-loaded electrospun PVA fiber mat (denotes as "neat") and the fiber mats that were cross-linked with the vapor from the aqueous solution of a) glutaraldehyde or b) glyoxal at various exposure time intervals as determined by transdermal diffusion through a pig skin method.

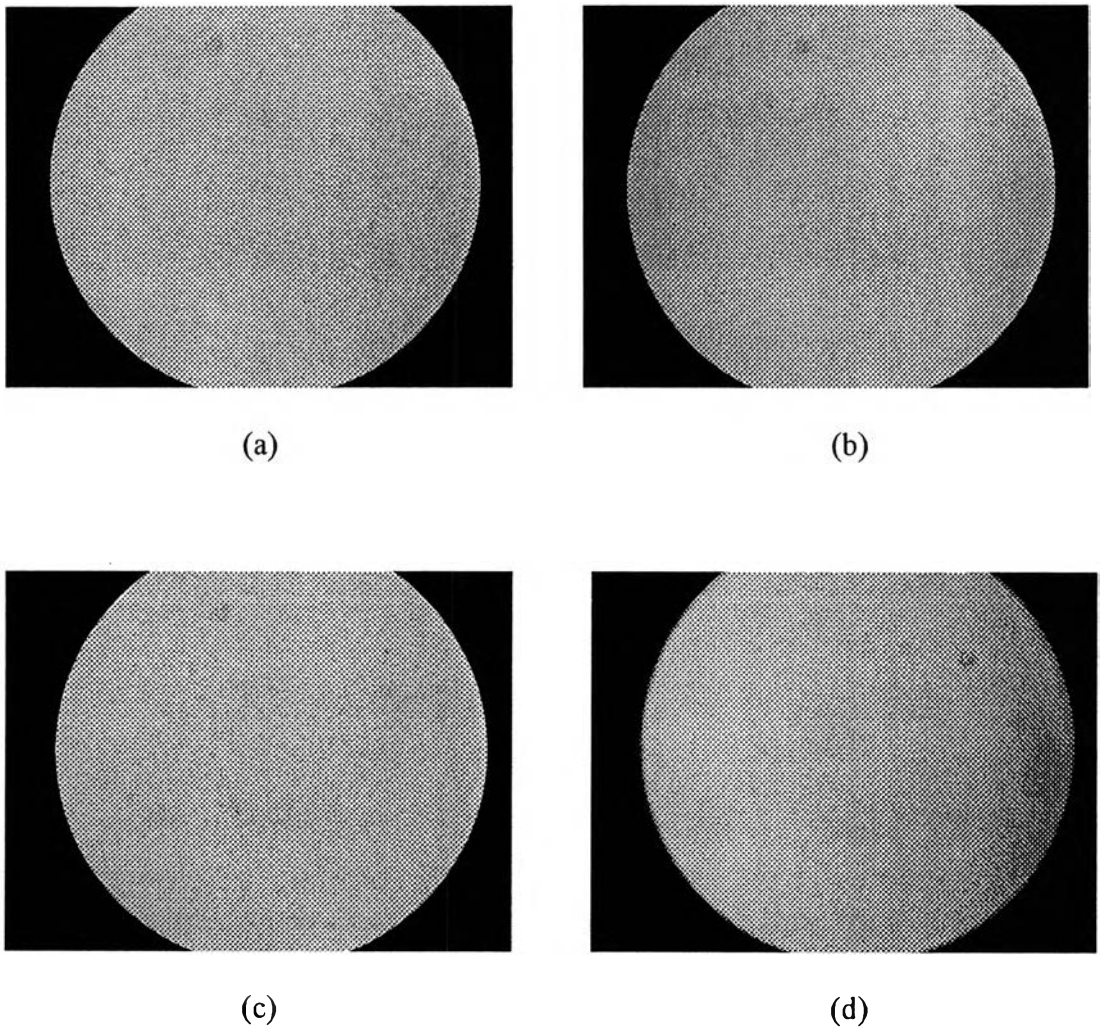


Figure 5.6 Morphology of human dermal fibroblast cells that were cultured on the surfaces of the (a) control, (b) SS-loaded e-spun PVA fiber mats, the SS-loaded e-spun PVA fiber mats cross-linked with (c) glutaraldehyde vapor for 5 h and (d) glyoxal vapor for 8 h.