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Contributed Paper

Effect of Degree of Acetylation on *In Vitro* Biocompatibility of Electrospun Cellulose Acetate-Based Fibrous Matrices

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ABSTRACT

Ultrafine fibrous matrices of regenerated cellulose (RC) of varying degrees of acetylation were facilely prepared from alkaline hydrolysis of electrospun cellulose acetate (CA; acetyl content ≈ 40%) fiber mats. The alkaline treatment was carried out in 0.1 N NaOH solution in 4:1 v/v water/ethanol mixture at 25 ± 1°C for periods of up to 24 h, after the CA fiber mats had been thermally treated at 208 °C for 1 h. After having been treated in the NaOH solution for 10 min, 30 min, and 24 h, the resulting CA-based fibrous matrices exhibited the acetyl contents of about 24, 11, and 4%, respectively. Indirect cytotoxicity evaluation of these CA-based fibrous matrices against human fibroblasts (HFF) and human keratinocytes (HaCaT) indicated that they posed no threat to the cells. The direct culture of the cells on their surfaces suggested that these fibrous matrices only supported the short term culture of both types of cells (i.e., 1 d), while the neat and the 24 h-alkaline treated CA-based fibrous matrices exhibited marginally good support for the proliferation of HaCaT.

Keywords: Electrospinning, Cellulose acetate, Regenerated cellulose, Biocompatibility.

1. INTRODUCTION

Electrospinning has been a subject of intense research, due to its ability to produce ultrafine fibers, with diameters in the range of tens of nanometers to less than ten micrometers, that exhibit high surface area to volume/mass ratios [1,2]. The principle of the process is the use of electrical as opposed to mechanical force as the main driving force for fiber formation [1-6]. Morphology of the electrospun fibers depends on contributions from solution properties, process settings, and ambient conditions [1-6]. Due to the morphological uniqueness, the proposed uses of the electrospun fibers in the field of biomedical applications are, for examples, as carriers/substrates for enzyme encapsulation/

immobilization [7,8], carriers for drug delivery [9,10], and scaffolds for tissue regeneration [11-13].

Cellulose is one of the most common biopolymers on earth and is the primary structural component of cell walls of green plants [14]. Its usefulness has been known since the ancient time [14]. In the last decade, utilization of cellulose in biomedical applications was well documented [15-20]. Among these, many facets of biocompatibility were studied [15-18]. It has been proposed for uses as substrates/scaffolds for bone [16,18,19] and cartilage [20] tissue engineering. However, the fabrication of cellulose is hampered by its limited solubility in common solvents and its subservient to thermal degradation prior to melting. As a result, only a few reports on successful electrospinning of cellulose are available in the open literature [21-23]. Alternatively, ultrafine cellulose fibers can be obtained facilely from deacetylation of electrospun fibers of cellulose acetate (CA)- a cellulose derivative that is readily soluble in a number of organic solvents [24,25].

In the present contribution, ultrafine RC fiber mats were prepared from alkaline treatment of electrospun CA fiber mats. Various degrees of acetylation of the fiber mats were achieved by varying the treatment conditions. To fathom the possibility of using these CA-based membranes as topical wound dressing materials, biocompatibility of the native electrospun CA fiber mats and the ultrafine RC fiber mats of varying degrees of acetylation was assessed *in vitro* with human foreskin fibroblasts (HFF) and human keratinocytes (HaCaT).

2. EXPERIMENTAL DETAILS

2.1. Materials

Cellulose acetate (CA; white powder; M_w = 30,000 Da; degree of acetyl substitution ≈ 2.4; acetyl content = 39.7%) was purchased from Sigma-Aldrich (USA). Sodium hydroxide (NaOH) was purchased from Ajax Finechem (Australia) and hydrochloric acid (HCl) was from J.T. Baker (USA). Acetone (Carlo Erba, Italy), *N, N*-dimethylacetamide [DMAc, Labscan (Asia), Thailand], and ethanol [Labscan (Asia), Thailand] were used as-received.

2.2. Preparation of Electrospun CA Fiber Mats

The electrospinning of CA was carried out based on the method described in a published work by some of us and another colleague [26]. Briefly, a weighed amount of CA powder was dissolved in 2:1 v/v acetone/DMAc to obtain a CA solution at a fixed concentration of 17% w/v. Electrospinning of the solution was carried out using a Gamma High Voltage Research ES30P high voltage dc power supply as the power source, a flat-tipped 20-gauge stainless steel needle (OD = 0.91 mm) as the nozzle, and an aluminum foil wrapped around a home-made rotating metal drum (OD = 9 cm) as the fiber collection device. The electric field was 17.5 kV/15 cm, the rotational speed of the rotating drum was 60 ± 5 rpm, and the feed rate of the solutions was 1 mL h^{-1}. For 9 h of continuous electrospinning, the thicknesses of the CA fiber mats were 20-30 μm.

2.3. Thermal Treatment and Alkaline Treatment

The CA fiber mats were peeled off from the aluminum foil, sandwiched between two polytetrafluoroethylene (PTFE) sheets, and put in a hot-air oven at 208°C for 1 h [25]. They were then immersed in 0.1 N NaOH solution in 4:1 v/v water/ethanol mixture at room temperature (25 ± 1°C) for varying time intervals, ranging from 10 min

to 24 h. The alkaline-treated CA fiber mats were then rinsed in deionized (DI) water and kept in a desiccator prior to further uses.

2.4. Characterization

2.4.1. Physical characteristics

Morphological appearance of the neat, the heat-treated, and the alkaline-treated CA fiber mats was observed by a JEOL JSM-5200 scanning electron microscope (SEM). They were sputtered with a thin layer of gold using a JEOL JFC-1100E ion sputtering device prior to SEM observation.

2.4.2. Chemical functionalities and degree of deacetylation

Chemical functionalities of the neat and the alkaline-treated CA fiber mats were investigated by a Nicolet Nexus 670 Fourier-transform infrared spectroscope (4 cm^{-1} resolution with 4 scans over the wavenumber range of 400-4000 cm^{-1}). The degree of deacetylation (*%DD*) of the alkaline-treated CA fiber mats was evaluated by a titration method. Specifically, weighed samples of the CA fiber mats (2.8 cm in diameter) were immersed in 0.1 N of NaOH solution in 4:1 v/v water/ethanol mixture at the ratio of 1:1 w/v. After 10 min to 24 h of immersion, 5 mL of the NaOH solution was pipetted out and then titrated with 0.1 N HCl aqueous solution, with phenolphthalein being used to indicate the basicity-to-acidity crossover. The amount of the hydroxyl ions participated in the deacetylation reaction of CA could then be calculated from the amount of the titrant, which was used to calculate the *%DD*, i.e.

$$\%DD = \frac{m_i - m_f}{m_i} \times 100 , \quad (1)$$

where m_i and m_f are the moles of the acetyl groups in the CA fiber mats before and after the alkaline treatment.

2.4.3. Wide-angle X-ray diffraction studies

The crystalline nature of the neat, the heat-treated, and the alkaline-treated CA fiber mats was examined by a JEOL JDX 3530 wide-angle X-ray diffractometer (30 kV and 40 mA over the 2Theta range of 5 to 50°).

2.4.4. Physico-chemical characteristics

The water retention and the loss in the mass upon submersion in water of the neat and the alkaline-treated CA fiber mats were measured after their submersion in distilled water at 37°C for 24 h. If M_i denotes the initial mass of each fiber mat sample in its dry state and M and M_d are its masses after submersion in distilled water in its wet and dry states, respectively, the property values can be calculated as follows:

$$\text{Water retention (\%)} = \frac{M - M_d}{M_d} \times 100 , \quad (2)$$

$$\text{Mass loss (\%)} = \frac{M_i - M_d}{M_i} \times 100 . \quad (3)$$

2.5. Biological Evaluation

The potential for use of the neat and the alkaline-treated CA fiber mats as wound dressing materials was assessed by the indirect cytotoxicity evaluation and the direct cultures of mammalian cells onto their surfaces. Two types of cells were used: 1) human foreskin fibroblasts (HFF) and 2) immortalized non-tumorigenic human keratinocytes (HaCaT). The experiments were done in adaptation from previously-published protocols [12]. Briefly, either type of cells was first cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA), supplemented with fetal bovine serum (FBS; Biochrom, UK), L-glutamine (Invitrogen, USA), penicillin (Gibco®, Invitrogen, USA) and streptomycin (Gibco®, Invitrogen, USA). When the cells reached 80% confluence, they

were trypsinized and counted by a hemacytometer, prior to further uses.

The indirect cytotoxicity of the fibrous materials was assessed based on the method that was adapted from the ISO10993-5 standard test method. Briefly, the specimens (~ 14 mm in diameter), pre-washed with 70% ethanol for 30 min, were washed with autoclaved phosphate buffer saline (PBS) solution twice and once with the culture medium. Either type of cells was seeded in wells of a 24-well tissue-culture polystyrene plate (TCPS; Corning, USA) at 2×10^4 cells/well. After 24 h of attachment, the culture medium was replaced with serum-free medium (SFM; DMEM containing penicillin and streptomycin, but without FBS). Each of the specimens was then place into each well and both the cells and the specimen were incubated further for 1, 3, and 5 d. The extraction ratio was fixed at 10 mg mL^{-1}. After each specified time interval was reached, the medium in each well was removed. The viability of the cells was then quantified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viabilities of the cells that had been cultured with fresh SFM were used as control.

For the direct cell culture studies, the well-washed specimens were placed in wells of 24-well TCPS. A stainless steel metal ring (12 mm in diameter) had been placed on top of each fiber mat specimen and 500 mL of the culture medium had been pipetted into each well, prior to the cell culturing. Subsequently, 2×10^4 cells/well of either type of cells were seeded onto each specimen and allowed to attach and proliferate for 1, 3, and 5 d. After rinsing the specimens with PBS twice to remove unattached cells, the viabilities of the cells were quantified by the MTT assay and the viabilities of the cells that had been cultured on TCPS were used as control. Lastly, the morphology of the cultured cells was investigated. The cell-cultured specimens were harvested and washed with PBS twice. After the cells had been fixed with 3% glutaraldehyde aqueous solution and washed with 0.2 M PBS aqueous solution, the specimens were dehydrated through a series of graded ethanol solutions and pure ethanol for 2 min and finally dried in air. Finally, they were observed by SEM.

2.6. Statistical Analysis

All the quantitative values were expressed as means ± standard deviation values. Statistical comparisons were performed using one-way ANOVA with SPSS 13.0 for Windows software (SPSS, USA). P values < 0.05 were considered statistically significant ($n = 3$).

3. RESULTS AND DISCUSSION

3.1. Morphology

The as-prepared 17% w/v CA solution in 2:1 v/v acetone/DMAc was electrospun under the electric field of 17.5 kV/15 cm for a fixed collection time of about 9 h [26]. A selected SEM image of the obtained fiber mats is shown in Figure 1a. The diameters of these individual fiber segments were determined to be 265 ± 39 nm ($n \geq 50$). Due to the fluffiness of the obtained fibrous matrices, further treatment in an alkaline aqueous solution would become a problem, as the materials could easily lose their physical integrity. Ma et al. [25] suggested that this shortcoming could be solved by thermally-treating the fiber mats at 208°C for 1 h. Figure 1b shows the morphology of the representative heat-treated fiber mat, which is obviously similar to that of the untreated precursor. The thermal treatment not only imparted the ease of handling to the fibrous matrices, but also helped

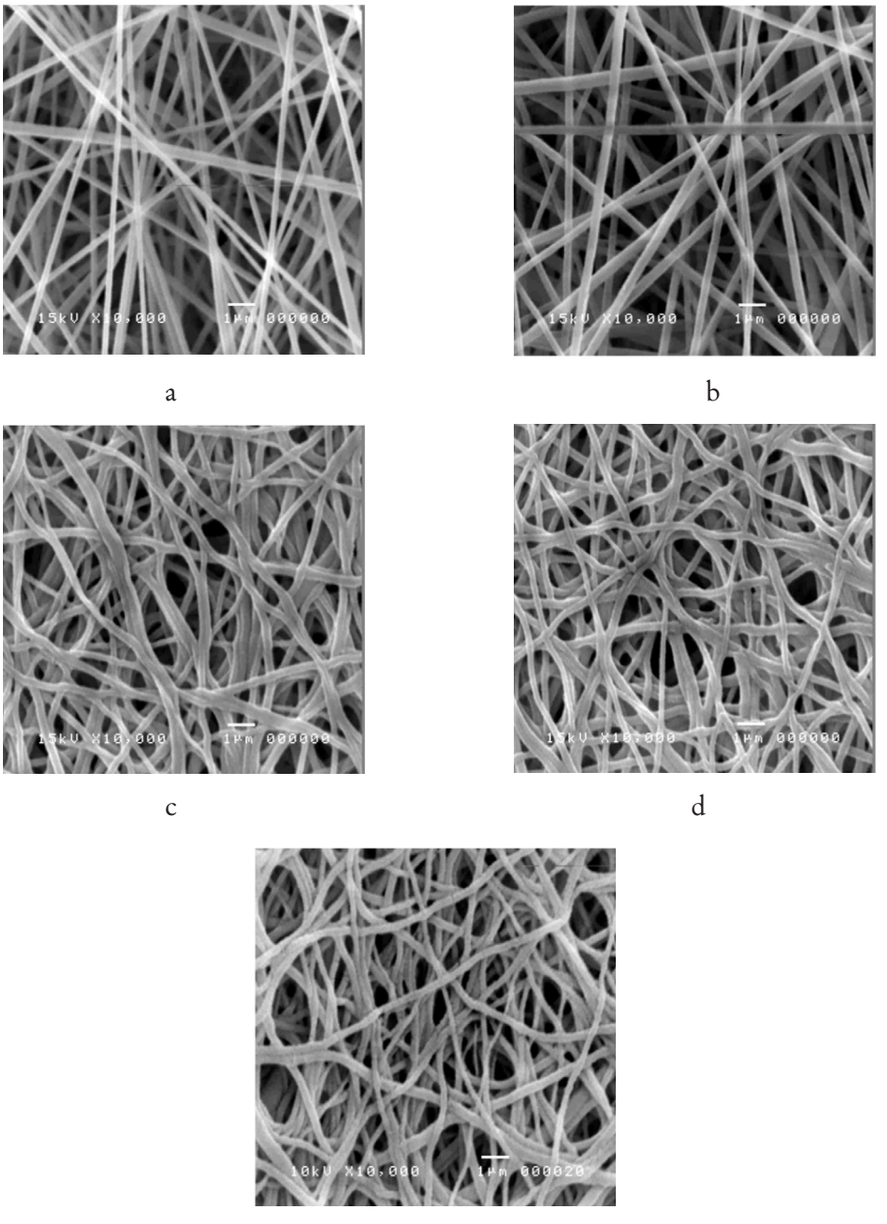


Figure 1. Selected scanning electron micrographs (magnification = 10,000x; scale bar = 1 μm) of electrospun fiber mats from 17% w/v CA solution in 2:1 v/v acetone/DMAc (a) before and (b) after heat treatment at 208°C for 1 h, including those of the thermally-treated fiber mats that had been submerged in 0.1 N NaOH solution in 4:1 v/v water/ethanol mixture at room temperature (25 ± 1°C) for (c) 10 min, (d) 30 min, and (e) 24 h. The electrospun CA fiber mats were fabricated under the electric field of 17.5 kV/15 cm for a fixed collection time of about 9 h.

maintain their physical integrity after having been submerged in 0.1 N NaOH solution in 4:1 v/v water/ethanol mixture at 25 ± 1°C for periods of up to 24 h. Figure 1c-e shows the morphology of the fiber mats after they had been submerged in the alkaline solution for 10 min, 30 min and 24 h, respectively. Apparently, the fibrous character of the alkaline-treated fiber mats was still intact, with no evidence of the individual fibers being destroyed. On the other hand, Son et al. [24] reported that, after being submerged in a potassium hydroxide (KOH) solution in ethanol for 30 min, obvious destruction of the CA fiber mats was evident.

3.2. Chemical Functionalities and *%DD*

The deacetylation reaction of the alkaline-treated CA fiber mats was followed qualitatively by FT-IR and quantitatively by titration. Figure 2 illustrates representative FT-IR spectra of a neat CA fiber mat and some of the CA fiber mats that had been submerged in the alkaline solution for 10 min, 30 min, and 24 h. For the neat CA fiber mat, a strong absorption peak centering at 1750 cm^{-1}, corresponding to the carbonyl groups (C=O) of the acetyl esters of CA, was evident. After 10 min of the alkaline treatment, the intensity of this peak decreased appreciably. Further increasing the alkaline treatment time resulted in a monotonous decrease in the intensity of the peak. The peak disappeared completely after submerging the mat in the NaOH solution for 24 h, thereby confirming the successful formation of RC fiber mats. In a similar manner, the presence of the peak centering at 1232 cm^{-1}, corresponding to the C-O stretching of the ester/ether groups of CA, decreased in its intensity upon submersion in the alkaline solution and even disappeared completely after 24 h of submersion in the alkaline solution. At 24 h of submersion in the NaOH solution, the presence of the peak

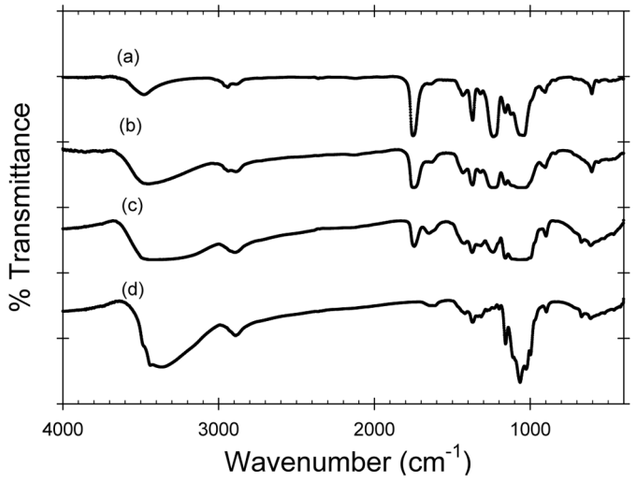


Figure 2. Representative FT-IR spectra of (a) a neat electrospun CA fiber mat and some of the thermally-treated fiber mats that had been submerged in 0.1 N NaOH solution in 4:1 v/v water/ethanol mixture at 25 ± 1°C for a period of (b) 10 min, (c) 30 min, and (d) 24 h.

centering at 1068 cm^{-1}, corresponding to the C-O stretching of the alcohol groups of cellulose, was clearly visible. Strikingly, the peak associated with the O-H stretching, centering at 3480 cm^{-1}, became more intense and broader for the alkaline-treated CA fiber mats, especially for the ones that had been submerged in the alkaline solution for 24 h.

The *%DD* values of all of the alkaline-treated CA fiber mats were determined by titration and the results are shown in Figure 3. It should be noted that the degree of acetyl substitution and the acetyl content of the as-received CA were 2.4 and 39.7%. The titration procedure was to quantify, in percentage, the amount of the acetyl groups that had been abstracted by the treatment with the NaOH solution. According to the obtained result, the *%DD* increased sharply from about 40% after 10 min of submersion in the alkaline solution to about 79% after 60 min of submersion. Further increase in the submersion time interval only resulted in a slight increase in the *%DD* to finally reach plateau values of about 88-90% after the CA fiber mats had been submerged in the alkaline solution for 7 to 24 h.

3.3. X-ray Diffraction

Figure 4 shows the X-ray diffraction patterns of the neat, the heat-treated, and some of the alkaline-treated CA fiber mats. For the neat CA fiber mat, two peaks at the 2Theta values of about 10.0 and 17.4° were observed. These peaks correspond respectively to the crystalline domains of CA and cellulose (ca. the acetyl content of the as-received CA was 39.7%). The intensity of these peaks increased appreciably after the thermal treatment, most likely a result of the increase in the mobility of the CA chains during the thermal treatment, hence the increase in the crystallinity of the crystalline domains. Kamide and Saito [27] reported a relationship between the glass transition temperatures (T_g, °C) and the degrees of

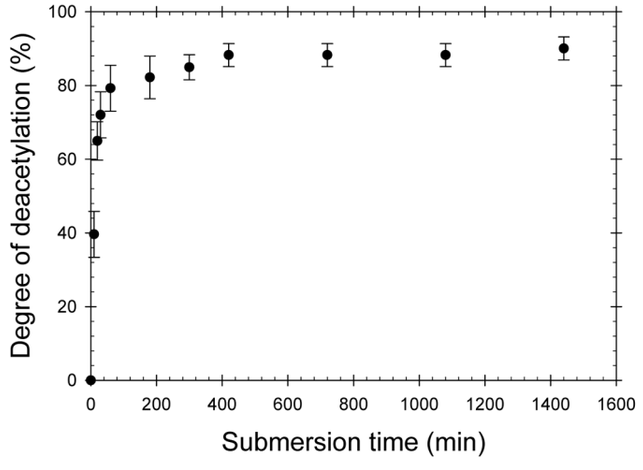


Figure 3. Degrees of deacetylation (*%DD*) of thermally-treated electrospun CA fiber mats that had been submerged in the NaOH solution as a function of submersion time (n = 5).

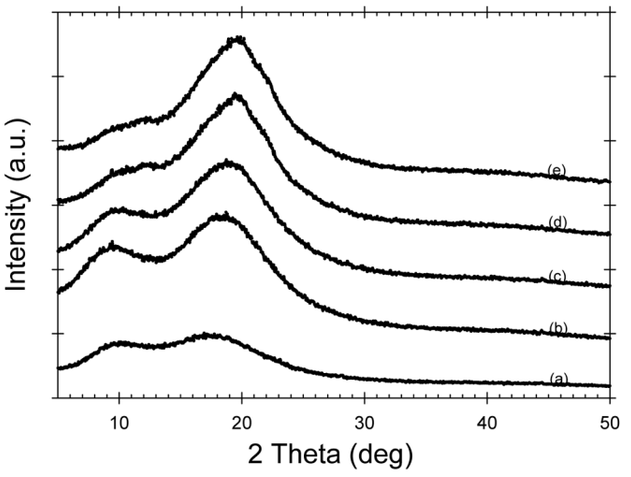


Figure 4. X-ray diffraction patterns of electrospun CA fiber mats (a) before and (b) after thermal treatment at 208°C for 1 h and those of the thermally-treated fiber mats that had been submerged in the NaOH solution for (c) 10 min, (d) 30 min, and (e) 24 h.

acetyl substitution ($z, \in [0,3]$) of CA, which was given as $T_g = 249.9 - 20.3z$. For $z = 2.4$, T_g of the as-received CA should be about 201°C. At the treatment temperature of 208°C, CA molecules should, therefore, have enough mobility to form more stable crystallites, hence the observed increase in the intensity of the crystalline peaks. The increase in the crystallinity of the thermally-treated CA would increase its stiffness and, at the same time, reduce the subservience to penetration by chemicals, hence an improvement of its physical integrity.

As for the alkaline-treated CA fiber mats, while the intensity of the low-angle peak decreased and shifted towards a higher 2Theta value with an increase in the treatment time interval, that of the high-angle peak increased and also shifted towards a higher 2Theta value. As the intensity of the high-angle peak increased with an increase in the alkaline treatment time, it becomes obvious that the decrease in the acetyl content along the CA chains was responsible for the observed increase in the crystallinity of the alkaline-treated CA fiber mats as well as the rearrangement of the crystalline packing into that of cellulose. The reappearance of the-OH groups, hence the occurrence of the hydrogen bonding, should be responsible for the observed increase in the crystallinity of the alkaline-treated CA fiber mats.

3.4. Physico-chemical Characteristics

The neat CA fiber mats and the CA fiber mats that had been treated with the alkaline solution for 10 min, 30 min, and 24 h were studied further. Based on Figure 3, the acetyl contents of these fibrous materials were estimated to be about 40, 24, 11, and 4%, respectively. These materials were tested for the water retention and the loss in the mass upon submersion in water for 24 h and the results are summarized in Table 1. The water retention decreased after the CA fiber mats underwent the alkaline treatment and the

Table 1. Water retention and the loss in the mass of thermally-treated electrospun CA fiber mats before and after submersion in the NaOH solution for 10 min, 30 min, and 24 h (n = 5).

Sample	Water retention (%)	Mass loss (%)
Neat	420 ± 19	0.6 ± 0.5
10 min of alkaline treatment	335 ± 13	1.0 ± 0.7
30 min of alkaline treatment	326 ± 25	1.1 ± 0.7
24 h of alkaline treatment	336 ± 16	1.4 ± 0.6

* These fiber mat samples had been submerged in distilled water at 37°C for 24 h.

variation in the submersion time did not have a strong influence on the property values. It should be noted that the retention of water in an ultrafine fibrous material originates from the absorption of water within the mass of the material and from the retention of water between inter-fibrous pores due to the capillary action. While the latter should increase when the CA fiber mats had been treated with the NaOH solution (i.e., due to the increase in the hydrophillicity), the fact that the water retention values of the alkaline-treated CA fiber mats were lower than that of the neat materials should be a result of the decrease in the absorbed amount of water within the mass of the matrix material. This, in turn, resulted from the fact that the CA fiber mats had to be thermally treated prior to being treated with the NaOH solution. The thermal treatment caused the crystallinity of the matrix material to increase, hence less amount of water to be absorbed. On the other hand, after the alkaline treatment, the loss in the mass of the fiber mats increased very slightly. It is well-known that cellulose undergoes alkaline degradation, the degradation occurs from the reducing ends of the molecule and produces water-soluble *D*-glucoisosaccharinic acid as a by-product [28].

3.5. Indirect Cytotoxicity Evaluation

The indirect cytotoxicity of the neat CA fiber mats and the CA fiber mats that had been treated with the alkaline solution for 10 min, 30 min, and 24 h were evaluated against HFF and HaCaT. Here, it is assumed that the viability of the cultured cells was proportional to the MTT absorbance. Figure 5 shows the viabilities of the cells that had been cultured in SFM both in the absence and in the presence of the fiber mat specimens for 1, 3, or 5 d were reported relatively to that of the cells that had been cultured in the absence of the specimens for 1 d. Evidently, the viabilities of both types of cells that had been cultured on the surface of TCPS, without or with the presence of the fiber mat specimens, increased monotonically with an increase in the cell culturing time. In fact, the viabilities of the cells, at any given cell culturing time point, were statistically the same, without or with the presence of the fiber mat specimens. The results indicate clearly that the proliferative ability of the cultured cells was unaffected, despite the presence of the fiber mat specimens in the culture medium of up to 5 d. In other words, these fibrous materials released no substances in the levels that would be harmful or pose any adverse effect to the growth in the number of the cells.

3.6. Cell Culture Studies

As previously mentioned, the acetyl contents of the neat CA fiber mats and

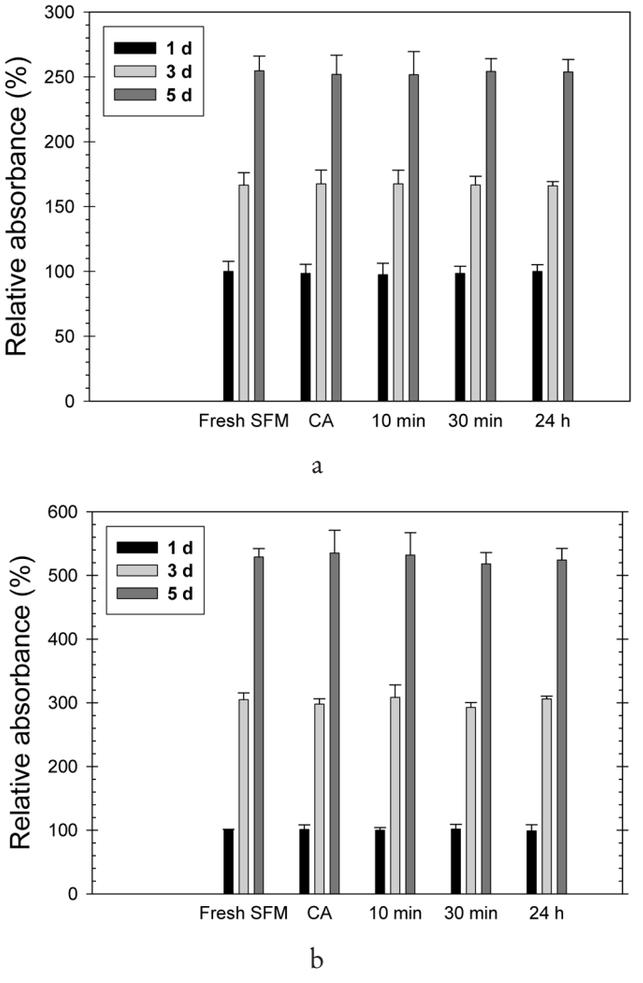


Figure 5. Indirect cytotoxicity evaluation of thermally-treated electrospun CA fiber mats before and after submersion in the NaOH solution for 10 min, 30 min, and 24 h, against (a) human foreskin fibroblasts (HFF) and (b) immortalized non-tumorigenic human keratinocytes (HaCaT) (n = 3). The fiber mat specimens were placed into the wells, that had been pre-cultured with the cells at 2×10^4 cells/well for 24 h, in serum-free medium (SFM) for a period of 1, 3, or 5 d at the extraction ratio of 10 mg mL^{-1}. The viabilities of the cells were reported in terms of the absorbance values relatively to that of the cells that had been cultured in the absence of the specimens for 1 d.

the CA fiber mats that had been treated with the alkaline solution for 10 min, 30 min, and 24 h were about 40, 24, 11, and 4%, respectively. These fibrous materials were tested further for *in vitro* biocompatibility with HFF and HaCaT. The cells were directly seeded and cultured on these fibrous matrices for 1, 3, and 5 d and those seeded and cultured on bare wells of TCPS were used as positive control. Again, the viability of the seeded and the cultured cells was proportional to the MTT absorbance.

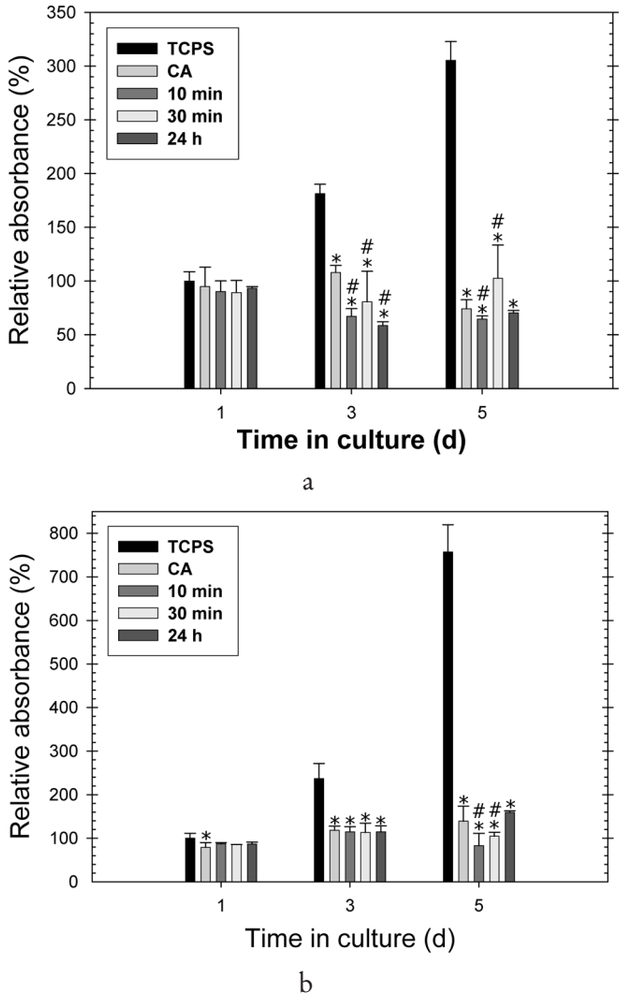


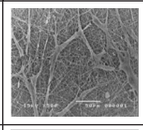
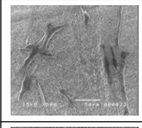
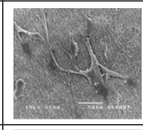
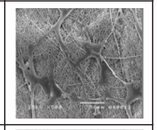
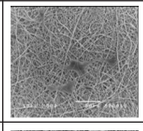
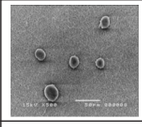
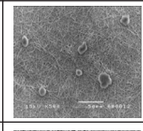
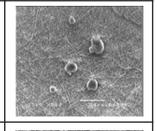
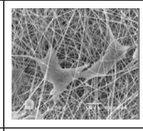
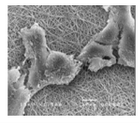
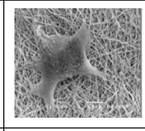
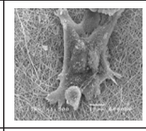
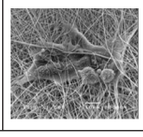
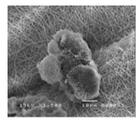
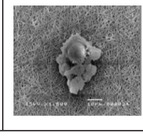
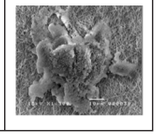
Figure 6. Attachment and proliferation of (a) HFF and (b) HaCaT that that had been seeded at 2×10^4 cells/well and cultured on tissue-culture polystyrene plate (TCPS, used as positive control) and thermally-treated electrospun CA fiber mats before and after submersion in the NaOH solution for 10 min, 30 min, and 24 h as a function of culturing time ($n = 5$). The viabilities of the cells were reported in terms of the absorbance values relatively to that of the cells that had been cultured on TCPS for 1 d. (*) $p < 0.05$ compared with TCPS at each specific time point and (#) $p < 0.05$ compared with CA at each specific time point.

Evidently, both types of cells adhered and proliferated well on the surface of TCPS. On day 1 of cell seeding, the viabilities of both types of cells that had been seeded on all of the fibrous matrices were not significantly different from those on TCPS, except for those of HaCaT that had been seeded on the neat CA fiber mats showing significantly lower values. On days 3 and 5, the viabilities of both types of cells that had

been cultured on all of the fibrous matrices were inferior to those on TCPS.

For HFF, the variation in the acetyl contents of the CA-based fibrous matrices did not pose strong effect on the viabilities of the cells cultured on them on day 1. Such an effect was seen when the cells had been grown for longer periods of time (i.e., 3 and 5 d). On day 3, only the neat CA fiber mats could support the growth of the cells particularly better than the alkaline-treated counterparts. On day 5, the viabilities of the cells grown on the CA fiber mats that had been treated with the alkaline solution for

Table 2. SEM images illustrating morphologies of HFF and HaCaT that had been grown on surfaces of thermally-treated electrospun CA fiber mats before and after submersion in the NaOH solution for 10 min, 30 min, and 24 h for 1 and 3 d.

Type of cells	Cell culturing time (d)	Type of CA-based fibrous matrices			
		CA	10 min	30 min	24 h
HFF*	1				
	3				
HaCaT**	1				
	3				

Magnification = *500x and **1500x

30 min increased, while those on the neat CA counterparts decreased, causing the 30 min-alkaline treated CA fiber mats to be the better support for the growth of the cells. For HaCaT, the variation in the acetyl contents of the CA-based fibrous matrices did not affect the viabilities of the cells grown on them on days 1 and 3, despite the slight increase in the viabilities of the cells on day 3, when compared with those on day 1. On day 5, the viabilities of the cells grown on the neat CA fiber mats and the CA fiber mats that had been treated with the alkaline solution for 24 h, which showed equivalent values, increased slightly from day 3, those of the cells grown on the CA fiber mats that had been treated with the alkaline solution for 10 and 30 min decreased slightly.

The morphologies of both HFF and HaCaT that had been seeded and cultured on the surfaces of the CA-based fibrous matrices are shown in Table 2. On day 1, both types of cells extended their cytoplasm over the surfaces of the fibrous matrices particularly well and even appeared in their normal cell shapes (i.e., spindle-like morphology for HFF and cobblestone morphology for HaCaT). On day 3, HFF on the neat CA fiber mat still appeared

expanded over the surface, but the extent of the cellular expansion was much decreased, when compared with the cells on day 1. HFF on the surfaces of the alkaline-treated CA fiber mats, on the other hand, were completely round. The change of the cell shape from spindle-like into round morphology, as the cell culturing time increased, suggested that the CA-based fibrous matrices are not supportive of the growth of human fibroblasts. This is in line with the observation reported by Sanchavanakit et al. [29] on bacterial cellulose fibrous membranes. Unlike HFF, HaCaT, on day 3 in their culture on the surfaces of all of the fibrous matrices, still retained their cobblestone morphology. However, the cells appeared to be more aggregated, especially those that had been cultured on the surfaces of the CA fiber mat that had been treated with the alkaline solution for 10 and 30 min. Despite the aggregation of the cells, the numbers of HaCaT on the surfaces of the CA fiber mat and the CA fiber mat that had been treated with the alkaline solution for 24 h increased from those on day 1. As reported by Sanchavanakit et al. [29], bacterial cellulose fibrous membranes showed good support for adhesion and proliferation of human keratinocytes.

4. CONCLUSIONS

FT-IR, titration, and X-ray diffraction confirmed partial removal of acetyl groups from the thermally-treated (208°C for 1 h) electrospun CA fiber mats upon submersion in 0.1 N NaOH solution in 4:1 v/v water/ethanol mixture at 25 ± 1°C. For the CA fiber mats that had been treated with the alkaline solution for 10 min, 30 min, and 24 h, the acetyl contents were about 24, 11, and 4%, respectively (cf. the acetyl content of about 40% for the as-received CA). The indirect cytotoxicity evaluation of the CA and the RC fibrous matrices against human fibroblasts (HFF) and human keratinocytes (HaCaT) indicated that these materials did not release any substance in the level that would be toxic or suppress the growth of both types of cells. Further evaluation with the direct culture of the cells on the materials revealed that these materials could only support the short term culture of both types of cells (i.e., 1 d) and only the neat CA fiber mats and the CA fiber mats that had been treated with the NaOH solution for 24 h showed marginally good support for the proliferation of the human keratinocytes. These results suggested the potential for use of the fibrous matrices as temporary dressings of skin wounds and this should be evaluated further *in vivo*.

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