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Comparison of Wound Morphology in Response to Electrospun PCL Nanofiber and Gauze Patches in Hamsters

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Abstract: *The unique properties of nanofibers that provided good air ventilation, moisture keeping and pathogenic barrier, made them suitable for wound dressing applications. In this paper, the wound healing process was compared between wounds treated with nanofiber and gauze dressings. Nanofibers were obtained from a fabrication of poly-(ε-caprolactone) (PCL) solution by electrospinning technique. In each hamster, two wounds were surgically excised of 5x5 mm, in which one wound was covered with nanofiber patch and the other was covered with gauze patch. The wound changes in gross lesion and histopathology were compared on days 4, 14 and 21 from 5 randomly picked hamsters at each time point. On day 4, the nanofiber patches produced a greater percentage of wound contraction when compared to the gauze patches (38.52% and 5.4%, respectively). More prominent progression of epithelium and new capillary formation were found in the wounds covered by nanofiber patches. There was not significantly different in the wound sizes of nanofiber and gauze patches on both days 14 and 21. However, the remaining of scar at the wounds covered with nanofiber patches was smaller compared to those of gauze patches. These results suggested that electrospun PCL nanofiber patches were suitable materials for wound dressing applications that facilitated faster wound healing process.*

Keywords: *PCL, Nanofiber, Gauze, Wound dressing, Hamsters, Electrospinning*

1. INTRODUCTION

Wound healing is the body natural process that immediately occurs after received lesions[1]. This process includes cooperative functions of many cells and released chemicals to repair a wound back to normal condition as much as possible [2]. One factor plays important role to wound healing process is oxygen, which involves the promotion of blood vessel growth, and the increase of fibroblast proliferation, collagen synthesis, and epithelial layer synthesis rate [1]. To receive a fast wound process, suitable

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wound dressing patch is required and it is the objective of many pharmaceutical products. Wound dressing patch should contain high porosity to allow oxygen exchange and be a good barrier to inhibit contamination of pathogens. Thus, materials for wound dressing must be selected carefully and tested for their efficiency and safety prior uses [3]. Gauze is one of commonly used wound dressings because it is good on liquid adsorption and air ventilation. It has been commercialized in many sizes and features, commonly made from cotton or synthetic fibers.

Nanotechnology has received increasing attention for many applications including nanometer-scale materials and devices for bio-medicinal applications such as stem cells [3], bone graft [4], artificial blood vessels [5], drug delivery [6], and wound dressing [7-9]. Among many techniques, electrospinning is one of feasible techniques to fabricate nanofibers from many natural and synthetic polymers by using high electrical voltages. With its diameter of nanometer, fabricated electrospun nanofibers has high surface area to volume ratio, which is more than 1:1000 fold compared to that of microfibers. Numbers of small pore sizes between non-woven interconnected nanofibers also provide good mechanical, electrical, and biological properties. Other properties such as strength, weight, and surface of nanofibers depend on polymer types [10, 11]. Many natural and synthetic polymers have been used to produced nanofibers. Natural polymers such as chitosan, silkworm silk, and natural rubber have been reported. In addition, many synthetic polymers that are biodegradable and biocompatible have been fabricated, including poly-(ε-caprolactone) (PCL) [12]. In this study, we are interested in studying the possibility of utilizing electrospun PCL nanofibers as wound dressing materials and compared its wound healing process to that of gauze dressing in hamsters induced skin wounds.

2. MATERIALS AND METHODS

2.1 Materials

Poly-(ε-caprolactone), chloroform, ethanol, and methanol were analytical grades and purchased from Sigma (Sigma, St. Louis, MO, USA). Isoflurane (Minradinc, PA, USA), paracetamol (T. O. Chemicals, Thailand), pentobarbitone (S.S.N.A. la Ballastiere, France), and enrofloxacin (Bayer, Germany) were also commercially available.

2.2 Experimental Animals

Fifteen female hamsters of 6-7 week olds were obtained from the Experimental Animal Center, Faculty of Medicine, Khon Kaen University. They were maintained at the animal house for 1 week to allow them adjusted to new environment. Single hamster was kept per cage. Animals were feed with food tablets and clean water. Cage bed was replaced every 4 days.

2.3 Fabrication of PCL Fibers

The fabrication of PCL fibers is similar to that reported in our previous study [12]. PCL fibers were fabricated using the KKU ElectroSys electrospinning unit (Department of Physics, Khon Kaen University). In a typical procedure, PCL solution was prepared as a 10 wt% in methanol:chloroform (3:1, v/v). Electrospinning parameters were set at a

distance of 13 cm, a voltage of 15 kV, and the spin rate of 1 ml/h. Ten ml of PCL solution was used to fabricate fibers, which was collected on an aluminum foil sheet. Electrospun PCL fibers were washed several times with sterile water, 75% ethanol, and dried in laminar flow hood before use.

2.4 Fiber Morphology

The morphology and diameter of electrospun PCL fibers were observed and determined from micrographs of scanning electron microscope (SEM) (LEO SEM1450VP, UK). Fabricated fibers of 1x1 cm were adhered to stubs and coated with gold by sputter coater for 3 min prior to observe under SEM. Diameter of PCL fibers were measured from 300 views and the average size was determined. The thickness of PCL fiber scaffold was measured by a micrometer. Its apparent density and porosity were calculated using following equations:

$$\text{Apparent density (g cm}^{-3}\text{)} = \text{mass of scaffold (g)} / [\text{thickness (cm)} \times \text{area (cm}^2\text{)}]$$

$$\text{Porosity (\%)} = [1-(\text{apparent density/bulk density})] \times 100\%$$

2.5 Wound Surgery

All procedures were approved by the Animal Ethic Committee of Khon Kaen University. Hamsters were deeply anesthetized by inhalation of isoflurane for surgical procedures. Two full-thickness excisional wounds of 5x5 mm reached a muscle layer were made on the left and right shaved back of hamsters. Electrospun PCL nanofiber and gauze patches were applied on the right and left wounds, respectively. Elastic fabric was applied on the hamsters to avoid scratch and lost of applied patches. Animals were given 0.1 ml paracetamol by oral administration everyday for 3 days and 0.1 ml enrofloxacin antibiotic twice a day for 6 days after the surgery to relieve pain and inhibit infection.

2.6 Gross Examination and Histopathology

Wounds of 5 randomly picked animals at 4, 14 and 21 days after surgery were examined. The mice were euthanized by isoflurane inhalation and pentobarbitone intraperitoneal injection and the wound with its surrounding tissue were dissected. Wound width and length were measured and calculated their wound areas. Wound healing percentage was calculated as the following formula:

Wound healing % = (present wound area/ original wound area) x 100.

Tissue in the wound area was excised as a piece of 1x1 cm and kept in 10% formalin. Samples were sent for histological analysis at Pathobiological laboratory, Faculty of Veterinary, Khon Kaen University. Inflammatory cell filtration, re-epithelialization, fibroblast growth, vascularization, granulation tissue, and collagen arrangement were compared between wound covered with nanofiber and gauze patches.

3. RESULTS AND DISCUSSION

3.1 Morphology of electrospun PCL fibers

Electrospun PCL fibers were produced from 10% PCL solution and its morphology was observed from SEM micrographs. The SEM micrographs showed the randomly

interconnected structure and smooth morphology of the PCL fibers, which was shown in Fig 1. The diameter of fibers was also counted and average size was 318.96±276.87 nm. The average thickness of PCL fiber scaffold was 145.8±6.2 mm. The porosity of PCL fiber scaffold was 76.50-77.86%, which was calculated from the known bulk density of PCL (1.145 g/cm^3) and the measured apparent density as shown in Table 1. Porosity was known as one of crucial parameter for wound healing process. Wound dressing scaffolds with porous nature would be beneficial for cellular infiltration, proliferation, sufficient gas and nutrient exchange, thus increasing wound healing rate. The preferred porosity of scaffolds should be in the range of 60-90% and the obtained electrospun PCL nanofiber scaffold showed its porosity in the range [13]. Nature of electrospun fiber scaffolds would contained high porosity that resulted from the space between randomly interconnected fibers, however, the well-defined pore sizes could not be easily control from these randomly deposited fibers. Nevertheless, high porosity of electrospun fiber scaffold would be beneficial for wound dressing applications.

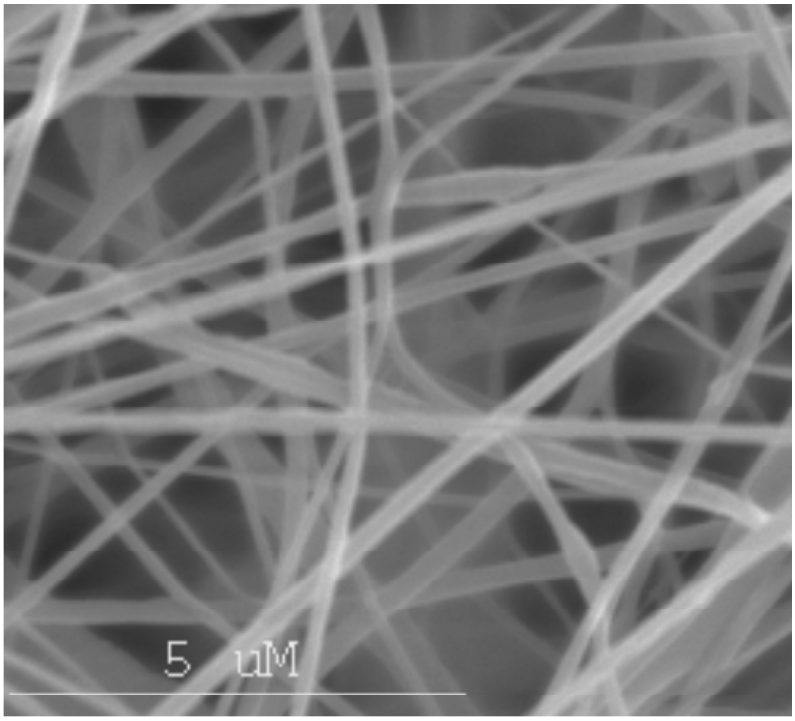

Figure 1: SEM micrograph of PCL nanofibers.

Table 1
Diameter, Thickness, Apparent Density and Porosity of PCL Fibers

Diameter (nm)	*Thickness (mm)*	*Mass per unit area (mg/cm^2)*	*Apparent density (g/cm^3)*	*Porosity (%)*
318.96±276.87	145.8±6.2	3.84±0.21	0.26±0.01	76.50-77.86

3.2 Gross Examination of Wound Contraction

In this study, we compared the wound healing process when electrospun PCL nanofiber and cotton gauze wound dressings were applied at induced wounds on hamsters. Two full-thickness excisional wounds of 5x5 mm were made on hamster backs and wound healing was observed on day 0, 4, 14, and 21. At each time point, five hamsters were randomly picked to observe wound sizes, which were determined as percentage compared to the wound sizes on day 0. The wound healing based on physical appearance as wound contraction and skin histology were studied. The physical wound contracted sizes on day 4, 14 and 21 were shown in Table 2. On day 4, nanofiber patches produced a greater percentage of wound contraction compared to that of gauze patches (38.52% and 5.4%). The average wound sizes of nanofiber and gauze patches were, respectively, 68.54% and 9.15%, suggesting a faster wound healing result of nanofiber patches.

Table 2
The Percentage of Wound Sizes on Day 4, 14, and 21 of Electrospun PCL Nanofiber and Gauze Covered Wounds in Hamsters

Day	*Hamsters*	*Wound sizes (%)*	
		Nanofiber	*Gauze*
4	1	78.58	90.48
	2	67.35	*
	3	76.52	100
	4	69.05	100
	5	52.39	86.11
	Average	68.54	94.15
14	6	25.00	44.23
	7	27.09	70.45
	8	0	13.33
	9	59.26	95.24
	10	*	*
	Average	27.83	55.81
21	11	39.29	91.30
	12	43.33	66.67
	13	42.59	42.86
	14	56.67	46.67
	15	46.94	69.39
	Average	45.76	63.38

* Could not measure due to adhesion of the wound dressing with the wound.

The wounds on day 14 and 21 were almost completely close. Remaining of scar, differed skin color from normal tissue, and newly grew hair were observed. There was not significantly different in the wound sizes between nanofiber and gauze patches on both day 14 and 21 (Table 2). However, the remaining of scar at the wounds covered with nanofiber patches were found smaller in sizes compared to those of gauze patches (Fig. 2).

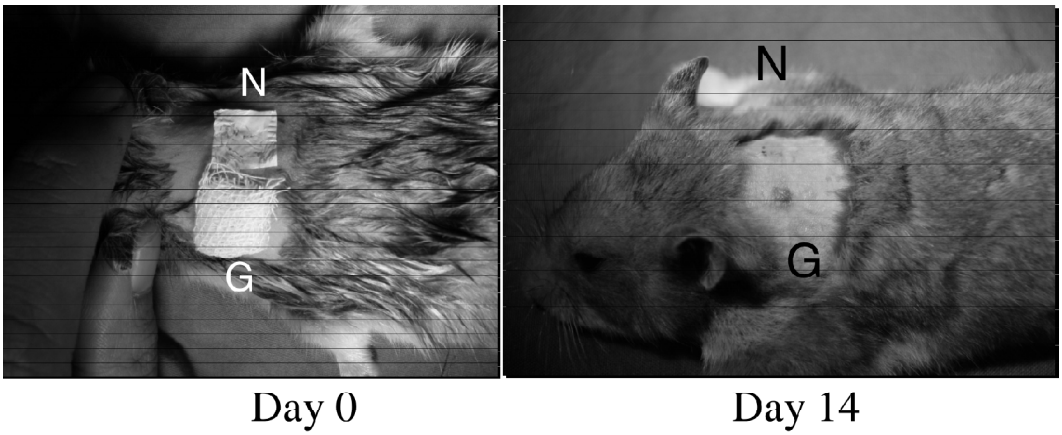

Figure 2: Wound morphology of hamsters on Day 0 and 14. After two excisional wounds of 5x5 mm were made on the back of a hamster, nanofiber (N) and gauze (G) dressing were applied on the right and left sides, respectively. On day 14, the wound of nanofiber dressing revealed a better healing morphology close to normal skin, while the wound of gauze dressing showed a more protruding granulation tissue.

3.3 Histological Changes

Tissues at wound sites were collected after determination of their sizes for histological studies. The results on day 4 showed on Fig. 3, which the histological changes of nanofiber and gauze applied wounds were similar overall except that more prominent progression of epithelium and greater newly capillarization were found in nanofiber covered wounds. The histological wound changes included acute inflammation response such as cell debris, necrotic cells at the borderline of wounds, dermal tissue hemorrhage and congestion were observed. The base and adjacent tissue of the wounds showed new capillary vascularization and inflammatory cells scattered infiltration. The active fibroblast and collagen fiber regeneration from the border of wound was observed.

Although, the histology of wounds covered with nanofiber and gauze patches were quite similar overall, it was noticeable that wounds covered with nanofiber patches contained more epithelium progression, fibroblast cell appearance, and new blood vessel formation, which were probably the reason of their faster healing process [14]. High porosity at nanometer scale of nanofibers would facilitate oxygen circulation, reduce accumulation of body fluid yet maintain moisture at wound site, and inhibit bacteria from external, thus causing a faster wound healing process [7]. In addition, non woven interconnection of nanofibers was suitable to cell adhesion, division, and proliferation, thus resulting in more epithelium growth. On the other hand, gauze had much larger fibers and pore sizes, therefore it did not had any properties to facilitate a fast healing, instead its larger pores could easily allow bacterial contamination from outside wounds [15, 16].

The skin epithelialization was almost completely recovered on day 14 in both types of wound dressing materials (Fig. 3). In nanofiber covered wound, the granulation tissue was well constructed in the organization of fibroblast and collagen fiber, less inflammatory

cell infiltration and reduced blood vessel formation. While, in gauze covered wound, there was more protruding granulation tissue and new blood vessels.

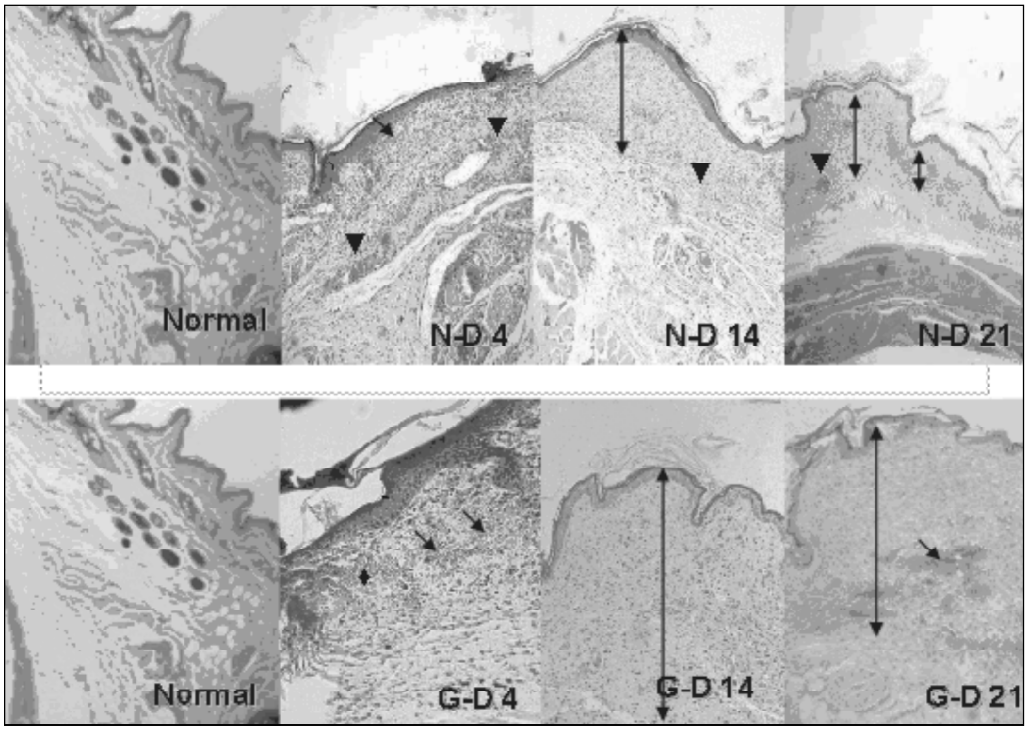

Figure 3: Histological changes of wounds at day 4, 14, and 21. Sections of tissues at wound sites were stained with haematoxylin and eosin dyes. Wounds were covered with either nanofiber (N) or gauze (G) patches. ▼: Blood congestion, ♦: Active fibroblast, ↓: Tissue hemorrhage, ↔: Dermal tissue, }: epidermal epithelialization.

On day 21, nanofiber treated wounds showed completely recovery when compared to normal skin but the gauze dressing wounds still had a thick granulation tissue, collagen fiber, inflammatory reaction and the formation of blood vessels (Fig. 3). It was interesting to point out that the observed scar in nanofiber treated wounds was smaller compared to gauze treated wounds. These might suggested that properties of nanofibers as mentioned before might facilitate faster wound healing and tissue arrangement [14].

Final goal of wound healing process was to obtain a fast heal with good quality, strength and morphology of skin both in inner and outer similar to normal skin as much as possible, which wound required many factors to accomplish these objectives. Normally, wound healing process would take 7-14 days, however, if it was disturbed or infected, longer wound healing time would be taken up to approximately 21 days [2]. Many factors were crucial to wound healing process. Sufficient amount of oxygen was important to stimulate blood vessel growth. Fibroblast proliferation, collagen synthesis, and increase rate of epithelialization would promote faster wound healing [1]. Completed synthesis

of granulation tissue gave good quality and strength of recovered wound, although not as much as the normal tissue but close to. In addition to oxygen, moisture was important to cell maintenance and proliferation, thus increase healing time, reduced size of scar, and minimized pain from small surgery [15]. Therefore, suitable wound dressing materials were important for wound healing process, which included allowing good air ventilation, fluid exchange, kept moisture and temperature at the wound sites, inhibit contamination and clean. In addition, they should be biodegradable and required no or less replacement that would interfere wound healing process. Properties of nanofibers, which met above requirements, thus were beneficial as wound dressing materials. In addition, nanofibers were feasible to incoporate biological active molecules, such as antibiotics, which was beneficial for wound healing process [6]. The results of this work showed a potential application of PCL nanofibers as wound dressing materials, especially in the aspect of fasten wound healing with less scar remaining.

4. CONCLUSION

In this study, the wound healing process in wounds treated with electrospun PCL nanofiber dressing was studied. Wound contraction and histological changes of wound treated with electrospun PCL nanofibers were compared to commonly used gauze dressing on day 4, 14, and 21. Wound treated with nanofiber patches showed a faster healing with less scar remaining. More epithelial progression and faster formation of good and strong granulation tissue were observed in wounds covered with nanofiber dressing. These results suggested that electrospun PCL nanofibers were beneficial and useful for wound dressing applications.

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