Characterization of Gelatin/CMC Scaffolds by Electrospinning and Comparison with Freeze Dry Techniques

Kaona Jongwuttanaruk, Prayoon Surin, and Fasai Wiwatwongwana

***Abstract*—Gelatin/CMC mixture was used to produce nanofiber scaffold in order to analyze fiber's physical characteristic. The gelatin solution made from dissolving gelatin in water which serves as solvent for the solution because water is a great solvent for gelatin. However, the solution used in fiber production with electrospinning, this research used organic solvent, 2,2,2-trifluoroethanol, which is also a good solvent for gelatin and hence produces good raw material for fiber production using electrospinning. However, since CMC was unable to dissolved under organic solvent like 2,2,2-trifluoroethanol, it was dissolved in water instead. The weight ratios of the gelatin/CMC mixture are 100/0, 90/10, 80/20, 70/30 and 60/40, respectively. Whereas higher or lower concentration of the solution resulted in failure to produce the fiber by using electrospinning. After nanofiber was produced, the size of the fiber was between 36-563 nm and this research also found that the scaffold of the 60/40 mixture occurred the smallest fiber with average fiber size of 41 nm which contained white granules in its structure. Water absorption analysis of fiber revealed that 100/0 mixture scaffold had the highest rate of absorption which was 11.79% and average lower swelling ratio of 62.7% in comparison with swelling ratio of the scaffold from freeze dry technique.**

***Index Terms*—Scaffold, Gelatin, carboxymethylcellouse, electrospinning, freeze dry.**

I. Introduction

Electrospinning is a fiber production method that can produce extremely small, in the order of nanometer, fiber from synthetic polymer or natural polymer. Electrospinning uses the principle of difference in potential [1] to deliver polymer to the collector. The size of the fiber is depended on the voltage, material's flow rate, distance between the injector and the collector for fiber and concentration of the material. The result of electrospun fiber can be in the form of non-woven fiber and aligned fiber. Electrospinning is popular among various industries, including in medicine such as for production of prosthetic skin and wound dressing material [2].

Mostly, production of fiber by electrospinning will use degradable polymer, and will focus on synthetic polymer such as PLA, PGA, PLGA and PCL as well as using natural polymer for medical application. This is because natural polymer can be easily accepted into human's body [3]. Gelatin is a type of natural biopolymer and can be extracted from collagen which is a substance that can be found abundantly in animal's skin, meat and bone. Gelatin also has another special characteristic such as highly biodegradable material and even though gelatin is hydrophilic polymer. It cannot be mixed and formed into fiber by electrospinning. Therefore, this research used a solvent that could efficiently dissolve gelatin, 2,2,2 trifluoroethanol with the weight/volume percentage concentration of 10 wt% [4].

However, because gelatin is quite expensive; in order to reduce the cost of prosthetic skin, it is necessary to mix gelatin with other cheaper natural polymer. Therefore, carboxymethyl cellulose or CMC is an interesting candidate in this research. It can improves the strength of prosthetic skin as well. This is because CMC is being used widely in many industries such as in industrial production of detergent, paint, glue, fabric, paper, ceramic, food and medicine. Moreover, CMC is colorless, odorless, does not present any harmful effect to human and a very efficient water soluble substance [5]. CMC or cellulose derivative is generally made of cellulose tissue that has high amount of alpha cellulose which is generally known as high quality cellulose and it is also cheaper than gelatin.

Moreover, in order to increase the fiber's mechanical strength, this research applied the crosslinking technique by using high temperature or dehydrothermal treatment (DHT). DHT is a physical process which concerns giving high thermal energy under vacuum to the material. Normally, this process used over 100 ^{o}C which extracted water from the fiber and cause condensation process that could produce the crosslinking of material and strengthens the fiber [6].

II. Experimental Details

A. Materials

Gelatin from Porcine Skin, 180 G Bloom, in powder form, Type B, from Fluka Analytical and 2,2,2-trifluoroethanol (TFE) (purity 99.0%) from Sigma-Aldrich were prepared. Preparation of gelatin solution was made by dissolving gelatin in TFE and stirred the solution at room temperature for 6 hours at the concentration of 10 %. Then powdered CMC

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from Sigma-Aldrich was dissolved in distilled water, at the concentration of 0.8 % and stirred at room temperature for 30 minutes. Higher or lower concentration of the solution resulted in failure to produce the fiber by using electrospinning. Afterward, the solution were prepared for fiber production using electrospinning where gelatin solution and CMC solution were mixed at the ratio of 100/0, 90/10, 80/20, 70/30 and 60/40 and then stirred at room temperature for 30 minutes.

B. Electrospinning

Gelatin/CMC solutions were prepared at the ratios of 100/0, 90/10, 80/20, 70/30 and 60/40 for fiber production by using electrospinning process was schematically shown in Fig. 1. The mixture was added into 5 ml syringe and the syringe was then attached to needle spinneret #20 with the hole size of 0.9 mm. The flow was controlled using syringe pump (KD-100, KD Scientific, Inc., USA) at the rate of 0.8 ml/h. High voltage electricity was supplied from high voltage power supply (RR501.25R/230/DDPM, Gamma High Voltage Research, USA) at 1 kV. The mixture was then injected onto collector which was made of foil sheet. The distance between the needle's end to the foil sheet was 13 centimeters.

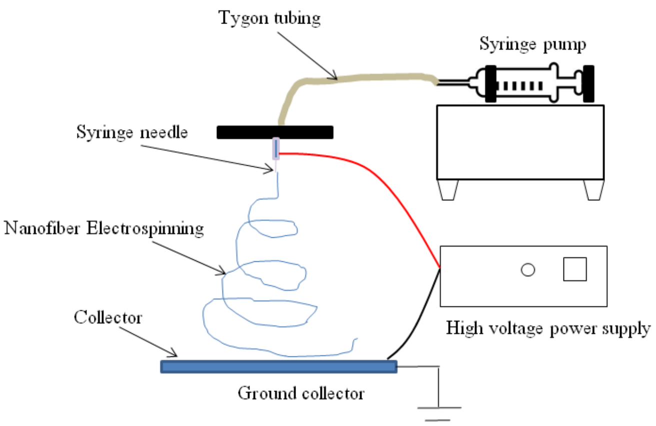

Fig. 1. Schematic diagram of electrospinning apparatus.

C. Dehydrothermal Treatment (DHT)

The resulted scaffold was then put into crosslinking technique by using DHT. The scaffold of gelatin and CMC mixtures at the ratio of 100/0, 90/10, 80/20, 70/30 and 60/40 was put into desiccator with silica gel in vacuum container for 2 days. Then the scaffold was put into vacuum oven under the temperature of 140 ^{o}C for 48 hours.

D. Characterization of Nanofibrous Scaffolds

1) Surface morphology of nanofibrous scaffolds

The scaffold was analyzed its morphology by using scanning electron microscopy (SEM, JSM-5610LV, JEOL) at the voltage of 20 kV after it has been coated with gold [7]. The fiber's thicknesses were also measured for at least 20 values and then calculated for the average thickness.

The morphology of the scaffold fabricated from electrospinning technique was shown in Fig. 2. When CMC was mixed into gelatin at different ratios, we found that the size of fiber and structure of scaffold was changed as well. The more CMC was added into the mixture, the smaller the fiber would be and CMC would be embedded into the fiber. In Fig. 2E which depicted the result of gelatin and CMC mixture at the ratio of 60/40, the fiber was quite small and contained several white granules all over the scaffold. Whereas the structure of scaffolds fabricated by freeze dry technique had porous structure. It was found that gelatin and CMC mixture at the ratio of 100/0, the porous of scaffold was quite small and the ratio of 80/20 showed the biggest pore [8].

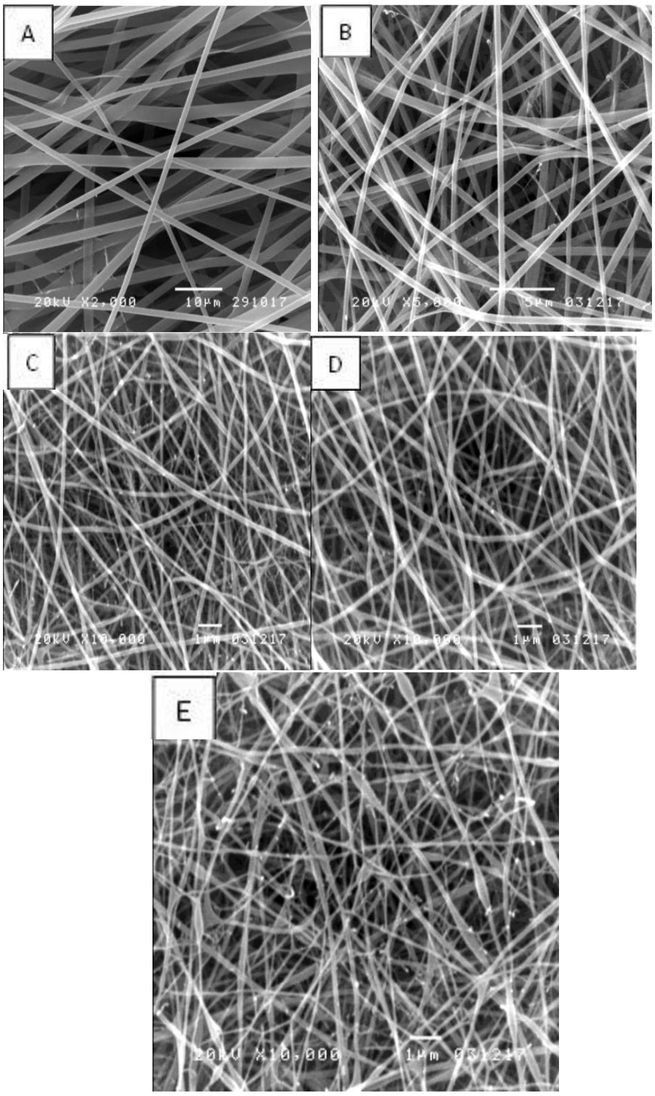

Fig. 2. SEM images of scaffolds for nanofibers electrospining with Gelatin/CMC ratios of 10/0 (A), Gelatin/CMC ratios of 90/10 (B), Gelatin/CMC ratios of 80/20 (C), Gelatin/CMC ratios of 70/30 (D), Gelatin/CMC ratios of 90/10 (E) and all condition were DHT for 48h.

Table I revealed that when CMC increased from 0 to 40 percent, fiber's size was decreased from 597 nm to 41 nm.

TABLE I: FIBER DIAMETERS OF ELECTROSPUN GELATIN/ CMC

Gelatin/CMC	Range of diameter (nm)	Average diameter (nm)
10/0	563-627	597
90/10	307-444	378
80/20	113-140	125
70/30	77-92	80
60/40	36-46	41

2) Swelling test

The scaffold was put into the swelling test in order to find the difference of dry weight and wet weight of the scaffold in percentage. The dried scaffold was weighted and then soaked the scaffold under PBS buffer solution at the pH of 7.4 and temperature of 37 °C for 3 hours [9]. Then both sides of the scaffold were wiped with low lint paper 10 seconds for each side and then weighted immediately. The dry weight and wet weight were then calculated into percentage using the following formula as shown in (1). We then compared the resulted of swelling ratio of scaffolds from electrospinning and freeze dry technique [10].

$$\text{Swelling ratio} = \frac{W_{so} - W_0}{W_0} \qquad (1)$$

where as

W_{so} is the weight of scaffold after its water content was absorbed

W_0 is the initial weight of the scaffold

Fig. 3 revealed that the scaffolds from electrospinning with the mixture of 100/0 ratio had swelling ratio of 11.79% which was the highest. All scaffolds from electrospinning had average lower in swelling ratio compared with the scaffolds from freeze dry technique, except for the case of gelatin and CMC mixture at the ratio of 90/10 which was higher than freeze dry technique. Meanwhile, the highest swelling ratio from freeze dry technique was from gelatin and CMC mixture at the ratio of 80/20 which was 44.67 % [11].

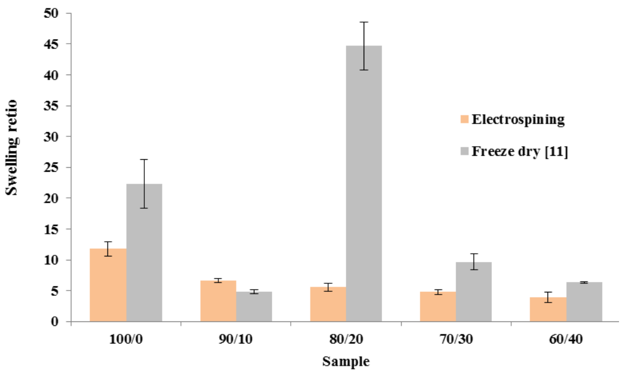

Fig. 3. Comparison of swelling ratio of Gelatin/CMC scaffold by Electrospinning and freeze dry.

III. Conclusion

Gelatin/CMC fiber produced by electrospinning technique revealed that the highest in swelling ratio and the largest of fiber size was found in gelatin and CMC mixture at the ratio of 100/0. Adding CMC into the gelatin fiber occurred in smaller in fiber size and lower in swelling ratio compared with pure gelatin fibrous scaffold. We could summarize that size of the fiber effected to the swelling ratio which the smaller of fiber, the lower of swelling ratio was found.

Analyzing the nanofibrous scaffold produced by using electrospinning technique, the gelatin/CMC fiber with large fiber size would also has high swelling ratio. In similar to the scaffold from freeze dry technique, the scaffold with the mixture of gelatin and CMC at the ratio of 80/20 had the largest pore in the structure and also showed the highest swelling ratio which was 44.67%. Comparison of fiber production using electrospinning and porous scaffold from freeze dry technique, the average swelling ratio of fiber from electrospinning was 62.7 % which lower than the scaffold from freeze dry technique.

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