Effect of Freezing-Thawing Cycles on Morphological and Swelling Potential

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ABSTRACT: ***The transdermal patch is a polymeric-based patch that contains a distributed bioactive ingredient that delivers therapeutic agents across the human skin surface at a constant rate. In this analysis, the dual layer PVA patch was prepared using a combination of freezing- thawing (F-T) and electrospinning techniques to research the effect of F-T cycles on the manufactured patch's morphological structure and swelling ratio. Using Scanning Electron Microscopy (SEM) and immersion of dual layer PVA patch for 24 hours in distilled water, the effect of F-T cycles on swelling potential as well as the morphological analysis of the patch was employed and characterized. The connection between PVA cryogel and PVA electrospun nanofiber membrane has been shown by the morphological structure of dual layer PVA sheet. The results revealed that the dual layer PVA patch had been successfully manufactured as the nanofiber membrane under layer PVA electrospun does not dissolve completely in the F-T phase. In addition, it is shown in this study that the increase in F-T cycles has decreased the swelling potential of the dual layer PVA patch. It also found that the presence of PVA electrospun nanofiber, due to the high surface ratio of the electrospun nanofiber membrane, has also affected the swelling capability of the dual layer PVA patch. The highest percentage of swelling ratio for dual layer PVA with 3 cycles (2L-3C) was found to be close to 66 percent, while for dual layer PVA with 5 cycles (2L-5C) the percentage was found to be slightly lower (33 per cent). The enhancement of dual layer PVA patch can be used to test drug release and may also be a successful option for delivery of transdermal drugs.***

KEYWORDS: ***Poly-vinyl Alcohol (PVA), Electrospun Nanofibers, Freezing-thawing, Swelling Ratio.***

INTRODUCTION

PVA is a biocompatible water-soluble polymer of growing interest due to its large-scale applications as a biomaterial and for drug delivery systems, for the limited authorized construction of sensors and membranes. Of particular interest among existing PVA-based systems are the chemical and physical hydrogels with controlled macroscopic properties, as successfully demonstrated in biotechnological and biomedical applications. Electrospinning is the most cost-effective with simple tooling that uses electrostatic forces to manufacture polymer solution nanofibers with specific characteristics including wide surface area to volume ratio, ultrafine structure and high porosity with pore sizes varying from submicron to nanometre [1]. Additionally, the nonwoven, ultrathin, and super porous structures of fibrous electrospun films allow enough air and water vapour to permeate through. The development of electrospun nanofibers has been widely used for skin tissue scaffolds, wound dressings and drug delivery applications including Alzheimer's medicines, antimicrobial and antifungal medicines, enzymes, cosmeceuticals and genes. Properties of PVA cryogel prepared by cyclic F-T have been studied extensively and rely on a variety of factors, the most important of which are molecular weight, polymer concentration and number of F-T cycles [2].

Previous work by Butylina et al. has shown that the compressive properties of hydrogels have been adversely affected as the number of F-T cycles increases from three to five. Different variables such as time, temperature and number of cycles in the F-T process will affect the final properties of the cryogels, as well as the quality of polymer fractions. Many studied have found that the equilibrium-swelling ratio of PVA cryogels decreases as the number of F-T cycles increase [3]. A research by Peppas et al. reported that the crosslinks in cryogel impeded polymer chain mobility and resulted in a lower swelling ratio. The strongly swollen cryogel is a less compact structure and a smaller degree of crosslinking compared with the lower swelling rate of the cryogels. Increasing the number of F-T cycles contributes to more crystal formation that acts as cross-linking sites, thereby suggesting a more robust network structure that acts as a less swollen structure [4].

MATERIALS AND METHODS

Materials:

Poly (vinyl alcohol) (PVA, molecular weight ~ 89,000-98,000, 99+% hydrolyzed) was purchased from Sigma- Aldrich and distilled water as a solvent.

Fabrication of Dual Layer PVA patch:

To prepare a PVA solution at a fixed concentration of 10 percent w / v, a weighed amount of PVA powder was dissolved in distilled water at 80o C for 3 h. The solution was then refrigerated to room temperature (25o C). Electrospinning of the as-prepared solutions was performed by connecting the positive polarity emitting electrode from a Gamma High-Voltage Study high-voltage DC power supply to the solutions found in a regular 5-ml syringe. The open end of which was fastened to a blunt gauge-23 stainless steel needle (outer diameter = 0.91 mm), used as the nozzle, and the aluminium foil laminated collection plate (dimension = 15 cm x 15 cm) used as the fibre collection tool.

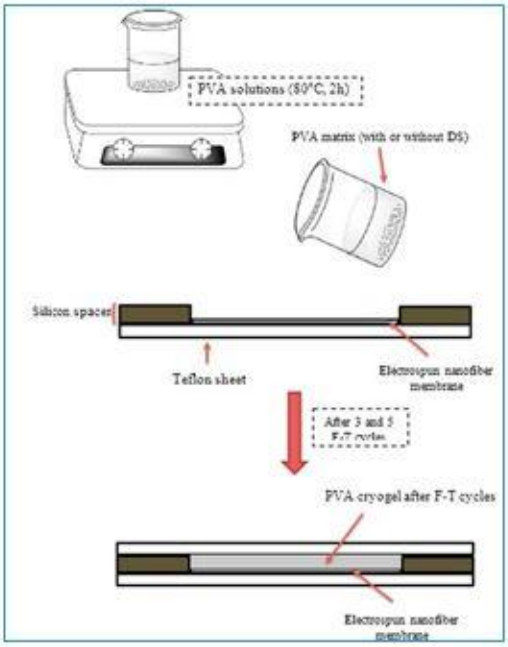

Figure 1. Schematic diagram of combine electrospun nanofiber membrane and F-T cryogel for 3 and 5 cycles.

A fixed electrical potential of 20 kV was applied between the tip of the nozzle and the outer surface of the collector plate (i.e. the electrostatic field strength of (20 kV/15 cm) over a fixed distance of 15 cm. The solution feed rate was regulated to approximately 1 ml h-1, using a syringe pump. The aqueous PVA solutions were then poured onto the electrospun nanofibers membrane surface which was placed within the specially built mould with dimensions: length x width x thickness: 100 mm x 100 mm x 1.5 mm. F-T of dual layer PVA patch was obtained by subjecting the PVA aqueous solutions with corresponding concentrations to repeated F-T cycles (3 and 5 cycles), consisting of a 24 h freezing step at -20 oC followed by a 2 h thawing step at room temperature. The choice of F-T period numbers was based on preceding studies. Figure 1 shows a schematic diagram of a dual layer PVA patch combination operation.

Scanning Electron Microscopy (SEM):

The freeze-dried dual-layer PVA samples were cut into small measurements (5 mm x 5 mm), and the samples were sent directly to Auto Fine Coater Machine for a sputtered thin layer of gold on its surface at 25 mA plasma current and 2 Pa chamber pressure to make the samples conductive. The coating purpose is to ensure that the freeze-dried isolating dual layer PVA samples are electrically conductive during high-resolution electron imaging applications. The Dual Layer PVA samples were then examined using JEOL-JSM6380LA (Japan) SEM, which operates under high vacuum at 15 kV at 10 and 50 μm magnifier.

Swelling property:

The pre-weighed dry samples were immersed at room temperature in distilled water for various periods before an absorption equilibrium was reached. After filter paper removed the excessive surface water the weight of the swollen gel was determined at different time intervals. The treatment was repeated until no further weight gain was detected [5]. The ratio of the swelling can be calculated as time function.

$$SR\ (\%) = \frac{W_t/W_o}{W_o}\ x\ 100 \qquad (1)$$

Where, W_t = Wet weight, W_o = Original dry weight.

RESULTS AND DISCUSSIONS

PVA electrospun nanofiber membrane:

Scanning Electron Microscopy, SEM is an excellent technique for examining the surface morphology of electrospun nanofibers and cryogels. Photomicrographs of electrospun nanofiber and average fiber diameter are shown in figure 2 (a) and (b).

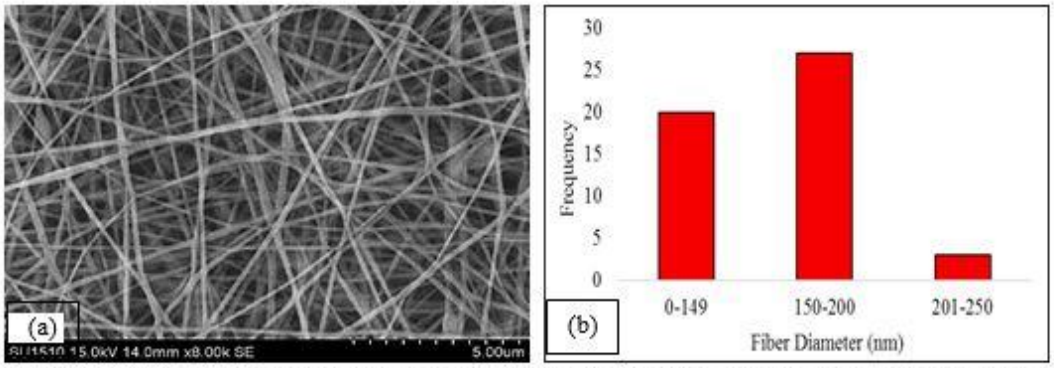

Fig. 2. SEM micrographs of average fiber diameter electrospun fibers from 10% PVA solutions (voltage = 20kV, tip–to nozzle distance =15 cm, flow rate = 1 ml/h). PVA magnification 8000x.

PVA Electrospun nanofibers with the correct electrospinning obtained smooth and uniform. The nanofiber has electrospinned successfully, and the selected parameters are consistent in the experiment for electrospinning [7]. The resulting fibres displayed fibre diameters of cylindrical morphology ranging from 90 to 250 nm as in figure 2 (b). The PVA electrospun nanofiber obtained was further produced with dual layer PVA patch.

Composition of Dual Layer PVA patch:

The process was stated in section 2.2 for preparation of dual layer PVA patch. An average thickness of PVA electrospun nanofiber membrane was measured at 5 different points after running 2 ml of electrospinning technique. Table 1 demonstrates how the dual layer PVA patch is constructed.

Table 1. Composition of Dual Layer PVA patch

Cryogel samples	Concentration of PVA (%w/v)	Thickness of electrospun nanofiber membrane (mm)	No. of cycles
2L-3C	10	0.062 ± 0.005	3
2L-5C		0.059 ± 0.009	5

Morphological of Dual Layer PVA Patch:

The combined technique (F-T and electrospinning) was carried out with success. Figure. 3. (A) and (b) show 2L-3C and 2L-5C morphological structures. After each F-T cycle is complete, PVA Cryogel (upper layer) and PVA electrospun nanofiber (bottom layer). After F-T cycles the mixture of PVA cryogel and PVA electrospun nanofiber does not demolish the nanofiber even though both structures were hydrophilic [8]. However, it does impact the diameter of the nanofiber as the nanofiber observed swollen in shape during the pouring process of PVA solution on top of nanofiber. The dual layer PVA patch has, on physical inspection, a phase distinction between gel and nanofiber. A cross-section of the dual layer PVA patch shown in Figure 4, may support this argument [9].

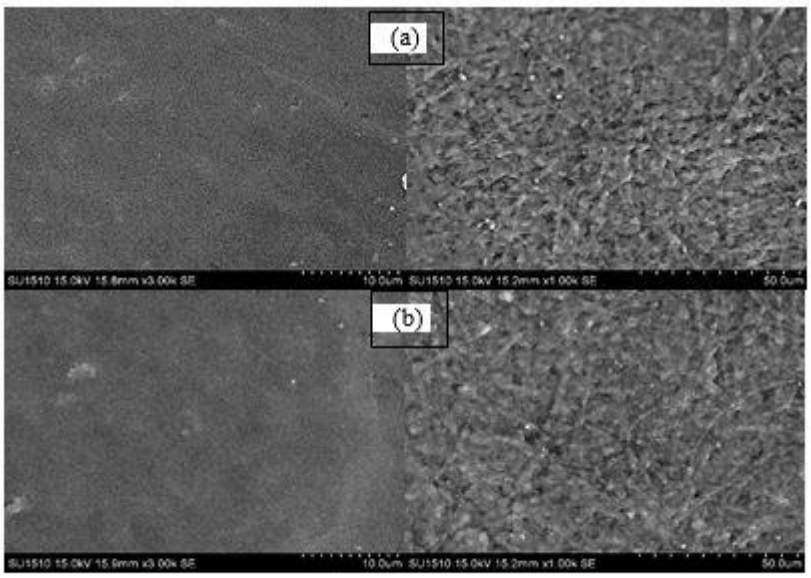

Figure 3. Surface morphological (top and bottom) of (a) 2L-3C and (b) 2L-5C.

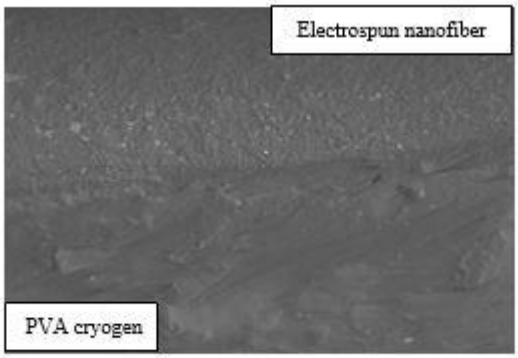

Figure 4. SEM micrograph for cross-section of dual layer PVA patch

Swelling Ratio:

Consult Figure 5, swelling ratio for both dual layer PVA patches submerged in distilled water as a 24-hour medium. Illustration. 5 Reflects 2L-3C and 2L-5C patches with swelling ratio.

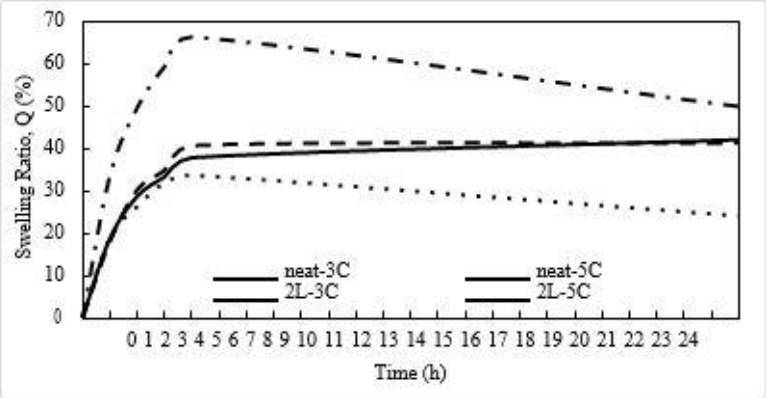

Figure 5. Swelling ratio of 3 cycles-dual layer PVA patch and (b) 5C-dual layer PVA patch.

Figure. 5 showed that 2L-3C had the highest swelling ratio of up to 66 percent compared to 2L-5C (33 percent) for the first four hours of immersion compared to 2L-5C. These findings suggest that the swelling activity is greatly affected by the amount of F-T cycles and nanofiber membrane incorporation in PVA cryogel. Previous work by Butylina et al. has shown that the compressive properties of hydrogels have been adversely affected as the number of F-T cycles increases from three to five. Different variables such as time, temperature and number of cycles in the F-T process will affect the final properties of the cryogels, as well as the quality of polymer fractions. Many studied have found that the equilibrium-swelling ratio of PVA cryogels decreases as the number of F-T cycles increase [10].

A research by Peppas et al. reported that the crosslinks in cryogel impeded polymer chain mobility and resulted in a lower swelling ratio. The strongly swollen cryogel is a less compact structure and a smaller degree of crosslinking compared with the lower swelling rate of the cryogels. Increasing the number of F-T cycles contributes to more crystal formation that acts as cross-linking sites, thereby suggesting a more robust network structure that acts as a less swollen structure. Essentially, as the number of freezing and thawing cycles increased, more PVA chains were involved in forming a stable 2L-5C crystal resulting in heavy hydrogen bonding, and subsequent physical crosslinking resulted in less sample dissolution [11].

The samples of PVA cryogel namely neat-3C and neat-5C have also been investigated to better understand the swelling properties of the dual layer PVA sheet. The dual layer PVA patch graph demonstrated greater swelling capacity relative to PVA cryogel (neat-3C and neat-5C). The 2L-3C samples show the highest percentage swelling levels while the lowest in neat-5C. The swelling ratio for 2L-5C and neat-3C is, however, almost equal to one another. This phenomenon occurred because of the presence of the nanofiber membrane which increases the percentage of its swelling. As stated by the previous researcher, due to the high surface area and porosity nature of the electrospun nanofiber, the swelling capability of electrospun nanofiber is higher compared to cast film.

CONCLUSION

The dual layer PVA patch was manufactured in this work using a combination of F-T and electrospinning method to determine the effect of F-T cycles on its morphological structures and swelling characteristics. Results show that the rise in F-T cycles has reduced its ability to swell. As for the dual layer PVA patch, the findings show that the presence of nanofiber has increased the percentage of swelling due to the nanofiber's large surface area resulting in the nanofibers soaking up the water. Nutshell, the engineered dual-layer PVA patch obviously enhanced swelling properties for further evaluation of drug release.

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