Comparison of the Anti-Fungal Effect of Electro-Spinning Drug-Loaded Nano-Fibers and Pad Dry Drug-Loaded Fabrics

Mehran Afrashi*, Dariush Semnani, Farzaneh Alihosseini, Parvin Dehghan, and Mehrnoush Maheronnaghsh

Abstract- **In this research, the anti-fungal effect of fluconazole-loaded polyvinyl alcohol (PVA) nano-fibers was compared with that of drug-loaded fabric (DLF) to be used in medical textiles and fibrous pads as a local drug delivery system. The drug-loaded nano-fibers (DLNs) were coated on the fabric surface using the electro-spinning process. The DLF was then prepared by means of the pad-dry method. The characterization of the samples was carried out by SEM, FTIR, and XRD tests. UV-V is spectrophotometry was also used to measure the drug release rate. The anti-fungal effect was studied by the disk diffusion method. The drug release test showed about 75% fluconazole release in 90 min and the disc diffusion test indicated that the DLF has more effect than the DLN due to the fast drug release.**

Keywords: **nano-fibers, candida albicans, vulvovaginal candidiasis, fluconazole, drug delivery system**

I. INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common infection in humans that is caused by *Candida albicans* [1]. About three quarters of all women will have VVC at their lifetimes [2,3]. Currently, there are two methods for the treatment of VVC including local and oral therapy. Nowadays the preferred treatment of such infections remains a topical azole treatment [4]. Fluconazole is a systemic azole antifungal drug and it is one of the most valuable antifungal agents. It has advantageous physical-chemical properties [5,6].

Study in drug-delivery field was centered toward the development of effective materials for drug loading and its delivery specifically to the targeted location at a controlled release rate. Designing a drug-delivery system requires several factors such as the retention of a drug property, route of administration, ability to target release, and biocompatibility [7]. Polymeric drug delivery systems are mainly intended to achieve a temporal and locational control of drug delivery [8]. These systems have many advantages compare to the conventional drug forms such as reducing toxicity by delivering them at a controlled rate and improving therapeutic efficacy. Recently, polymeric nano-fibers were used as a new device for these systems [9]. Nano-fibers with a high specific surface area have received attention due to their potential applications for medical usages and drug delivery carriers [10]. Polyvinyl alcohol (PVA) was used as a hydrophilic polymer for drug-loaded systems. The physical characteristics of PVA dependent on its method of preparation from the hydrolysis, or partial hydrolysis, of polyvinyl acetate. Nano-fibers of this polymer can be used for releasing biological and medical molecules [11].

For evaluation of *in vitro* susceptibility testing of antifungal agents, there are various methods such as broth micro- and macro-dilution, agar dilution, E test, colorimetric micro-dilution, and disk diffusion method [12]. However, recent efforts have been devoted to develop more simple agar-based methods including E-test and disk diffusion tests [13]. These tests are attractive and the usual applicable method for researchers, because they are simple, reproducible, and lack of requirements for specialized equipment [14,15].

The aim of this study is to investigate both the fluconazole-loaded nano-fibers and finished fabric due to side effects of oral usage of fluconazole in the treatment of vaginal candidiasis and inclusiveness of the disease such as headache, dizziness, drowsiness, stomach or abdominal pain, upset stomach, diarrhea, etc. The samples are assayed by scientific tests, for example, the disc diffusion method. This system has been made of electro-spun PVA nano-fibers containing the drug. Moreover, the proposed system can improve the efficiency of the anti-fungal agent.

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II. MATERIALS AND METHODS

A. Materials

To prepare the electro-spinning solutions, ethanol 46.07 g/mol with the purity higher than 99.9% and PVA powder 72000 g/mol were obtained from Merck (Germany). Fluconazole powder was prepared from Amin Pharmaceutical Co. Phosphate buffer solution was purchased from CMG Co. (Iran). Sabouraud dextrose agar was prepared from Merck (Germany). Furthermore, weft-knitted fabrics (cotton/polyester) were used as the substrate of electro-spun fibers and also it was used for pad drying.

B. Methods

B.1. Preparation the Nano-Fibrous Mats and DLFs

To prepare the PVA solution, distilled water has been used as a solvent. The solutions with constant concentrations 8 (w/w%) of PVA with different amounts of fluconazole rather to the PVA (0, 10, 20, and 30%) were prepared by the method explained in the previous work [16]. The electro-spinning process has been carried out by the solutions at a voltage of 14 kV, the distance of the needle to the collector of 15 cm and the rate of 0.26 mL/h. The electro-spinning apparatus was assembled at the faculty of textile engineering, Isfahan University of Technology. The cotton/PET substrate was coated with electrospun nanofibers.

Moreover, the fluconazole was loaded in the fabric structure using the pad-dry method. Initially, the fabric samples were disinfected by ethanol solution. The solutions with different concentrations of fluconazole (0, 10, 20, and 30%) based on the weight of fabric samples were prepared. Then, the fabric samples were immersed in the solutions for 10 min. The immersion time is considered short enough due to the low surface tension and high volatility of the ethanol. The samples were extracted from the solutions (adding on 100%) and were dried in ambient conditions.

B.2. Characterization of Samples

The morphology and diameter of the electro-spun fibers were examined by the scanning electron microscopy (SEM, Philips XL 30). Fiber diameters were measured by Digimizer software. The compatibility of the drug and polymer was observed using an IR spectrometer (Hartmann and Braun-MB100 made in Canada). For this purpose, very small pieces of nano-fibers were mixed with KBR powder. Then, the mixed powders were pressed to make a pellet. Measurements were taken in the range of 400-1800 cm^{-1}. X-ray diffraction patterns of samples were obtained by Asenware-AW-XDM300 diffractometer at 40 kV and scanned at 10–60 °C. Furthermore, the scope of scanning velocity was 0.5°/S [17].

B.3. Release Test

The amount of drug release rate was determined by using a UV–visible spectrophotometer (UV mini-1240, Shimadzu, Japan). First, 2000 ppm of drug solution was prepared as the standard solution for determining the calibration curve. Then, serial dilutions (5, 25, 50, 100, and 200 ppm) were prepared by diluting the stock solution with ethanol. The absorbance values of these solutions were measured at a λ_{max} (260 nm) for PBS solution as a blank. A certain weight of DLNFs was placed in PBS solution (37 °C) to determine the drug release of samples. The influence of PBS on the structure leads to swelling of nano-fibers. According to this mechanism, the drug was released from the nano-fibrous mat into the buffer solution. The release rate was determined by measuring the absorption of the drug in the solution.

The disk diffusion method was used to determine the sensitivity of the *Candida albicans* PTTC 5027 to the fluconazole according to CLSI M44-S2 protocols. For this test, a suspension of the *Candida* with a concentration of 0.5 Mac-Farland standards was prepared, which was spread on a plate containing Sabouraud dextrose agar with a sterile swab. Disk-shaped fabric samples containing different percentages of fluconazole were prepared. They were put on the inoculated culture media with the *Candida*. The plates were incubated at 37 °C for 24 h. After this time, the inhibited halos were measured around disk samples.

III. RESULTS AND DISCUSSION

A. Morphology and Structure of Samples

To obtain the suitable parameters, the nano-fibrous samples were observed by an optical microscope. During the process of electro-spinning, all the parameters that were involved in this process, including the concentration of polymer, applied voltage, flow rate and distance from tip to collector, were carefully optimized. The SEM images of samples are shown in Fig. 1. The desirable nanofibers were produced with the electro-spinning of the PVA solution. Table I shows an average of fibers diameter of samples (n=30). By increasing the drug amount in the polymer solution, concentration of the polymer solution is increased, which reduces the stretch ability of the fiber during electro-spinning [17].

B. Further Characterization

The infrared (IR) spectra of samples are shown in Fig. 2. According to this figure, FTIR spectrum of PVA has

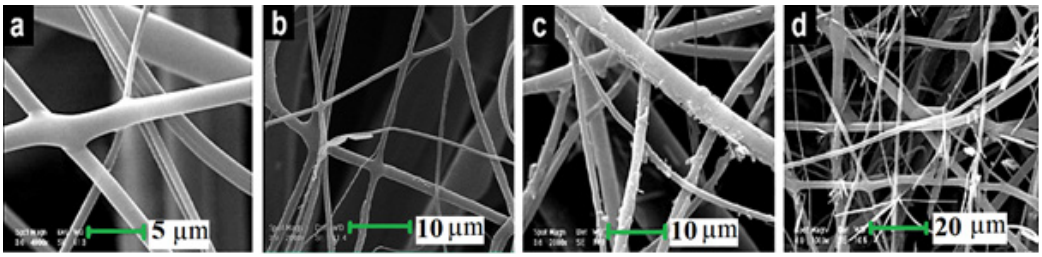

Fig. 1. SEM images of PVA fibers mat with different drug amounts. PVA fibrous mat: (a) without fluconazole, (b) with 10% fluconazole, (c) with 20% fluconazole, and (d) with 30% fluconazole.

TABLE I
AVERAGE AND SD OF THE FIBROUS SAMPLES DIAMETER

Sample	a	b	c	d
Mean (nm)	690	870	1040	1340
Std. dev (nm)	290	360	440	640

peaks at 1655 cm^{-1} relating to the bending band of HOH, at 2930 cm^{-1} related to the stretching bands of CH_2 and peaks at 1720 and 1575 cm^{-1} are related to the carbonyl group. Furthermore, peaks at 850 and 610 cm^{-1} represent stretching vibrations (C-C), that correspond with reports of other researchers [18]. It also can be seen that IR spectra of fluconazole correspond with reports of other researchers [19]. The FTIR spectrum of DLNFs shows the presence of fluconazole and PVA. Therefore, it can be concluded that there is no incompatibility between the drug and the polymer. XRD measurements determined the physical status of fluconazole in the PVA nano-fibers. These measurements indicated that the pure drug has a crystalline structure but it was converted into an amorphous due to load in the nano-fibers. As shown in Fig. 3, the presence of many different reflections in the XRD pattern of pure fluconazole shows that the pure drug is a crystalline material. Furthermore, the diffraction patterns of drug-loaded nano-fibers appeared at around 2θ=21° indicate the sample gets amorphous structure [20].

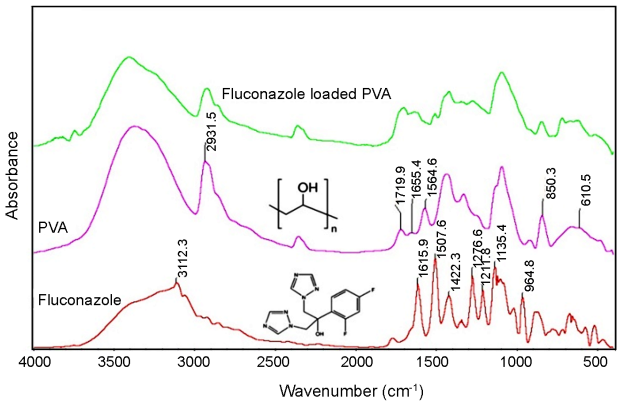

Fig. 2. Infrared (IR) spectra of PVA nano-fibers, fluconazole, and PVA nano-fibers containing fluconazole.

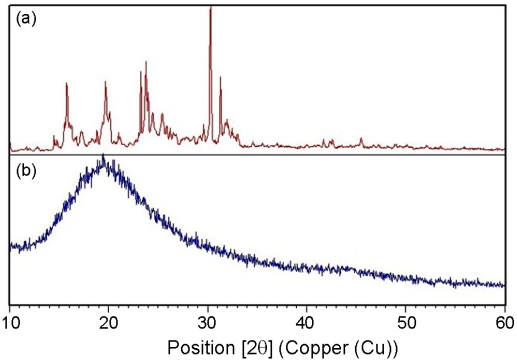

Fig. 3. XRD pattern of: (a) pure fluconazole and (b) fluconazole loaded PVA nano-fibers.

The XRD result confirms that fluconazole was highly dispersed in the PVA nano-fibers mat and was present in an amorphous mode where the original structure of the pure drug had been lost.

C. Release Test

By drawing the calibration curve of the serial dilutions of the standard solution, line equation (Eq. (1)) with R^2=0.997 was obtained.

$$Y = 0.0023x + 0.0176 \quad (1)$$

Where, x is the fluconazole concentration of the standard solution, and y is the absorbance of the UV–Vis spectrophotometer.

In this system, the drug release profile was evaluated. As can be seen in Fig. 4, the general trend of release diagram of the fluconazole loaded samples in the system can be found. According to this figure, the drug release is fast and about 75% of the drug released in the first 90 min from the start time of the release process.

The diameter of the fiber is effective on the rate and mechanism of drug release. The surface area increased by reducing the diameter of the nanofibers. Therefore, a higher area of fiber gets exposed to the release environment and the release rate increases. According to this test, the drug release is fast and about three quarters of drug trapped in

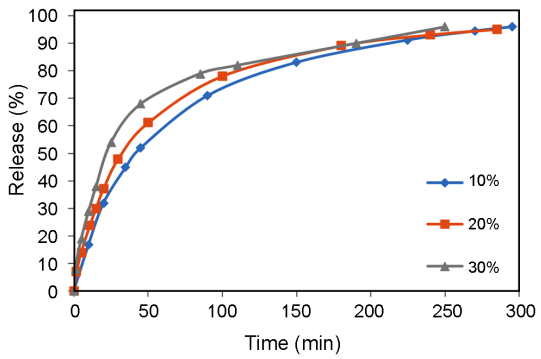

Fig. 4. Release diagram of the fluconazole loaded samples.

fibrous mat, which was released in the first 1.5 h from the start time of the release process. This fast release can be attributed to the drug on the outer surface of nano-fibers and a high specific surface area of nano-fibers. After it's fast release, the release rate was decreased and after about 6 h, the drug was released in the delivery environment. The general trend of release in samples containing different amounts of the drug was almost similar. Just it can be seen that by increasing the amount of drug samples, the initial release rate increases. The release rate of the drug in the system is related to solubility, degradation, swelling, and properties affecting the mechanism and rate of drug delivery. The probability of drug existence on the surface of fibrous mat increases by increasing the amount of drug in the polymer solution. This matter leads to suddenly increasing rate of drug release at the beginning of release process. For the treatment of this disease, the burst release is an advantage, because it is necessary to eliminate the fungus quickly. Due to its topical release, the side effects of the drug are reduced. The release time is controlled by some factors such as diameter, cross-linking and polymer percentage.

D. In-Vitro Test-Disk Diffusion Method

The drug-loaded samples have been shown in Fig. 5. According to this figure, the fungal has been grown around the fabric and this sample has no effect on destroying them. Furthermore, in Figs. 5a, 5b, and 5c, the fabric samples coated by 10, 20, and 30% nano-fibers have been shown, respectively. Therefore, the lack of growth of fungus to form a circular halo is visible around the samples. The average diameters of these halos have been measured 24.6, 27.4, and 33.4 mm, respectively. The samples have been taken from three different parts of the fabric. It can be found that the middle part of the fabric shows more anti-fungal effect than other parts of the fabric due to the uniformity of the electro-spinning of nano-fibers.

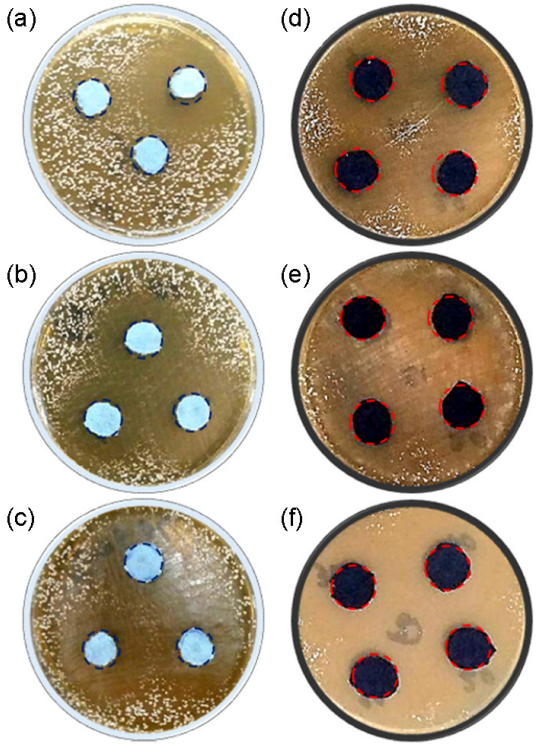

Fig. 5. *In vitro* effect of coated samples by fibrous mat containing: (a) 10%, (b) 20%, (c) 30% fluconazole; and drug pad-dry loaded fabrics containing (d) 10%, (e) 20%, and (f) 30% fluconazole.

It can also be seen the diameters of halos related to pad-dry samples are relatively more than those of drug-loaded samples by the electro-spinning method. The average diameters of these halos have been measured 26.1, 28.7, and 32.6 mm, respectively. The reason of different anti-fungal effect in two methods of drug loading can be demonstrated in the different quantity of drug loading in samples. Furthermore, in the pad-dry method, the fluconazole loading in the fabric is physical and drug molecules bind on the surface of fabric yarn and fibers. Therefore, as soon as one perches the sample in the release environment, drug molecules get out of fabric quickly.

IV. CONCLUSION

In this study, topical use of fluconazole was investigated with two methods through drug delivery systems. In one method, different amounts of fluconazole were added to the PVA solution and coated on the fabric by the electro-spinning method. In the other method, the fluconazole was solved in ethanol and the fabric samples were immersed in the solution. By optimizing the electro-spinning conditions, the nano-fibrous mat was prepared uniformly and without any beads. The results of SEM showed that the mean and standard deviation of fiber diameter were

increased by increasing the amount of fluconazole. The XRD measurements indicated the drug has a crystalline structure, however, the fluconazole-loaded nano-fibers have an amorphous structure. The release test indicated that the release rate of fluconazole is almost fast. The disc diffusion method showed the drug-loaded samples prevent the growth of fungus due to the release of fluconazole. Drug loading by pad-dry method is more effective than that by electro-spinning method (about two folds), but drug loading and release in the electro-spinning method are more controllable.

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