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DEVELOPMENT OF FLUCONAZOLE-LOADED POLY(VINYL ALCOHOL)/POLY(ETHYLENE GLYCOL) BLENDED FILM FOR TREATMENT OF CANDIDA-INFECTED CUTANEOUS WOUNDS

Peerapat Chidchai[1], Worranan Rangsimawong[1,2], Praneet Opanasopit[1,3], Prasopchai Patrojanasophon[1,3], Chaiyakarn Pornpitchanarong[1,3], Prin Chaksmithanont[3,*]

[1] Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom

[2] Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani

[3] Department of Industrial Pharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom

* Corresponding author: chaksmithanont_p@su.ac.th

ABSTRACT

In this work, we aimed to develop fluconazole (FLU)-loaded polymer blended film (FLU-film) for candidiasis wounds. The films were prepared from poly(vinyl alcohol) (PVA) and poly(ethylene glycol) 1000 (PEG 1000) mixed at different proportions by weight, casted on a petri dish, and dried at 60ºC for 12 h. Tensile strength, swelling ratio, and morphology were analyzed to determine an appropriate film for wound application. The selected film was loaded with 0.5% w/w FLU, where the drug homogeneity was ascertained. Then, the drug release profile, cytotoxicity of the films, and antifungal activity were determined. The findings revealed that the suitable film was PVA:PEG 1000 (95:5), showing anticipated flexibility and stretchability, as well as a soothing texture, while the swelling and molecular attributes were comparable to other films. FLU was homogenously loaded into the selected film and presented a prolonged release profile following Higuchi's model. The film was found to be non-toxic to the human skin fibroblast. Lastly, the FLU-film presented 1.16-fold greater antifungal effectiveness than the free FLU. Overall, the FLU-film has been developed and proven to be effective for treating Candida-infected wounds.

Keywords: fluconazole, poly(vinyl alcohol), poly(ethylene glycol), films, candidiasis

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Introduction

Wounds are damage to the integrity of biological tissues, including mucous membranes, organ tissues, and skin. Cutaneous wounds can be caused by cuts, falls, surgery, etc. An infected wound is a localized defect on the skin and the underlying layers in which pathogenic organisms have invaded into viable tissue of the wound. Upon wound infection, the body's immune response is triggered, leading to inflammation and, subsequently, cell damage. However, the healing process is delayed.[1] Mild wounds, even with infections, such as a scratch or infected hair follicle, can be self-contained and healed spontaneously. However, infections can become problematic if not properly treated with appropriate medical interventions.

Candida albicans, in particular, is an opportunistic human pathogen in which, when the conditions are ideal, it can become pathogenic or capable of causing diseases. Candida skin infections can affect nearly every part of the body. However, they are more common in intertriginous areas since warm, moist, and sweaty environments are ideal for the fungus to grow.[2] Clotrimazole, miconazole, fluconazole (FLU), itraconazole, and nystatin were some of the azoles and polyenes used to treat cutaneous candidiasis.[3] FLU, a broad-spectrum bis-triazole, molecular weight 306.27 g/mol, is well-known for its antifungal activities. FLU, a white crystalline powder, is slightly soluble in water with logP 0.5.[4] FLU exhibits efficacy against a wide range of fungal infections, including those induced by Candida species, rendering it a prevalent option for the management of candidiasis. FLU is commonly utilized in both systemic and topical forms because it has a high level of oral bioavailability, a positive safety profile, and a relatively low occurrence of negative effects.[5]

The commercial products for the treatment of candidiasis cutaneous wounds are cream, gel, and topical solution.[6,7] The commercial product has numerous disadvantages, including the requirement for frequent application, irregular absorption of the medication, and restricted duration of antifungal effectiveness. These constraints result in insufficient efficacy and inconvenience for patients. Moreover, the frequent use of these medications might lead to irritation and difficulty following instructions. To tackle these difficulties, there is an increasing demand for inventive drug delivery systems that offer long-lasting and efficient antifungal effects while reducing the need for frequent application and improving patient adherence.

A wound dressing film is a type of medical dressing used to cover and protect wounds, ulcers, or skin injuries. These films are designed to provide a sterile environment, promote wound healing, regulate moisture levels, and create an optimal environment for the healing process. Additionally, they can control the release of drugs, are painless to remove, and are suitable for use on various parts of the body.[8,9]

Polyvinyl alcohol (PVA) and polyethylene glycol (PEG) are two commonly used polymers in the formulation of wound-healing films. PVA is biocompatible, retains moisture, can absorb exudate, and adheres well to the wound.[10] Meanwhile, PEG is highly hygroscopic, has lubricating properties and improves the flexibility of the film.[11] When combined in a film formulation, they can have synergistic effects.

In this work, we proposed a swellable film as a dressing material that would facilitate the wound healing process by hydration and exudate adsorption with antifungal activity for candidiasis cutaneous wound treatment. Incorporating FLU into the polymer-blended film would be beneficial by improving drug wettability and drug dissolution, resulting in an improvement in antifungal activity. Poly(vinyl alcohol) (PVA) and poly(ethylene glycol) 1000 (PEG 1000) blended film loaded with FLU was developed. The film was fabricated based on PVA with varied PEG 1000 content, and the optimal film for cutaneous wound was selected for drug loading

and release examination. The FLU homogeneity was confirmed prior to the biocompatibility assessment. Notably, the antifungal activity of the FLU-loaded polymer blended film (FLU-film) was examined. The optimal ratio of PVA and PEG 1000 to form a suitable film for candidiasis cutaneous wound was established herein.

Methods and Methods

Materials

PEG 1000 was bought from Sigma Aldrich (St. Louis, MO. USA). PVA was procured from Merck & Co. (Darmstadt, Germany). FLU was kindly given from Bangkok Lab & Cosmetics, Co., Ltd. (Ratchaburi, Thailand). All other chemicals and solvents were used as received without purification.

Preparation of polymer blended film

The films were prepared using the solution casting method. They were composed of PVA and PEG 1000 in different weight ratios. PVA was prepared at 10% w/w in warm water (80ºC), and the PEG 1000 solution was prepared at 50% w/w. Each film was prepared from PVA with various percentages by weight to PEG 1000 (Table 1). The mixture was poured into the petri dish at an exact mass and dried in a hot air oven at 60ºC for 12 h.

Characterizations of polymer blended film

1. Tensile strength

The tensile strength of the films was determined using a TXXT Plus texture analyzer (Stable Micro Systems, Surrey, UK) with a 5-kg load cell. The films were sliced into 10×20 mm^2. Tensile stress, tensile strain, and Young's modulus were measured using tensile grips with a test speed of up to 5.0 mm/sec until film breakage.

2. Attenuated-total reflection infrared spectroscopy

The components of the polymer-blended films were confirmed using ATR-FTIR (Nicolet iS5, Thermo Fisher Scientific, MA, USA). The spectra were collected from wavenumber 4000–400 cm^{-1} using 4 cm^{-1} resolutions with 16 running scans.

3. Swelling Index

The PVA/PEG 1000 films with a dimension of 10×10 mm^2 were soaked in 15 mL phosphate buffered saline (PBS) pH 7.4 at room temperature. The initial weight of the films (W_0) was measured before soaking the film in PBS. The films were taken out for weighing at different time intervals until a constant weight was reached (W_i). The film was taken out at each interval, and excess water was removed with a paper towel prior to being re-weighed. The percentage of swelling was calculated using the Eq. (1)

$$\% \text{ Swelling} = \frac{W_i - W_0}{W_0} \times 100 \quad \text{---------- (1)}$$

Drug loading and homogeneity

The drug was loaded into the polymer mixture to contain 0.5% w/w FLU before drying. Briefly, FLU powder was accurately weighed, while the polymer solutions were mixed separately. Then, FLU was added to the polymer solution and mixed until homogeneous before drying. Then, the film was randomly cut from different locations (5×5 mm^2 at each location) to ensure drug homogeneity. The drug in FLU-film was extracted in 1 mL absolute ethanol for 48 h in a tube rotator. The solution was collected and measured by UV-spectroscopy at 260 nm using a multimode microplate reader (VICTOR NIVO™, PerkinElmer, USA). The percentage of drug loading was calculated using the Eq. (2)

$$\% \text{ Drug loading} = \frac{\text{Weight of drug in film}}{\text{Weight of the feed drug}} \times 100 \quad \text{--- (2)}$$

Cytotoxicity

The normal human skin fibroblast (HSF) cells (ATCC, Rockville, MD, USA) were cultured in fetal bovine serum (FBS) and antibiotic-supplemented Dulbecco's modified Eagle's medium (DMEM). The cells were seeded at 10,000 cells/well into a 96-well plate (Flat-Bottom Corning™ Costar™, Fisher Scientific, NH, USA) and incubated at a 5% CO_2 and 37ºC environment overnight. The blank film and FLU-film were prepared by dissolving 10 mg of the film (containing 50 µg of FLU) in DMEM, and they were

subsequently used to treat the cells for 24 h. Then, the samples were removed, and cells were rinsed using sterile PBS pH 7.4. Solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 0.5 mg/mL in DMEM was added and incubated for another 3 h to allow the formation of the formazan crystals. The absorbance of dissolved crystals by dimethyl sulfoxide (0.1 mL) was measured at 550 nm, and the relative percentage of viable cells was calculated.

Antifungal activity

Candida albicans (ATCC, Rockville, MD, USA) was prepared in Sabouraud dextrose broth (SDB) at a final concentration of 1×10^6 CFU/mL. Then, *C. albicans* was spread onto the surface of Sabouraud dextrose agar (SDA) using a sterile cotton swab. The FLU-film, weighing 10 mg, was formed into a circular shape and contained 50 µg of FLU. Meanwhile, FLU solution with an equivalent amount to the drug in the film was dissolved in deionized water and utilized as the positive control. Additionally, a blank film was used as a negative control. The clear zone diameters, which determine the antifungal efficacy of the formulation, were then measured after the agar plates were incubated at 37°C for 24 h.

Drug release

The FLU-film was immersed in 10 mL of PBS pH 7.4 in a shaker incubator at 37±2°C with a shaking speed of 150 rpm. During the release test, 0.5 mL of medium was collected at pre-determined time points for FLU analysis. An equal amount of fresh PBS was replaced in the container to maintain a constant volume. The collected samples were placed in a 96-well plate, and the FLU content was measured by UV-spectroscopy at 260 nm. A control solution was created by dissolving FLU powder in PBS pH 7.4 to create a 0.5% w/v FLU solution. This solution was then placed in a dialysis bag with a molecular weight cutoff of 6-8 kDa.

Statistical analysis

The data are expressed in mean ± standard deviation (S.D.). One-way ANOVA followed by post-hoc tests were performed to determine the differences that can be identified when p-value<0.05.

Results and Discussion

Characterizations of polymer blended film

All films were easily cast and provided smooth texture after drying. The films slowly turned more turbid once the amount of PEG 1000 was increased. Moreover, the thickness of the films was between 0.090 – 0.140 mm. From the tensile strength analysis (Table 1), it was found that the F1 film had the highest stress and the least strain, which is in concordance with its blister-like appearance. Once PEG 1000 was added to the film (F2-F5), the stress of the film was reduced, and the strain increased. Young's modulus, which is the measurement of the ability of a material to withstand changes in length under the lengthwise force of the films, suggested that, once PEG 1000 was incorporated, the film could be easily stretched and deformed compared to the film without PEG 1000. The analysis also presented that the F2-F4 films had similar stress, strain, and Young's modulus (p>0.05). However, the F5 film showed different stress and Young's modulus (p<0.05), which shows that the film was more flexible and could be stretched easily.[12,13] According to the results, the F1, F2, and F5 films were selected for further characterizations based on their mechanical property difference.

The ATR-FTIR analyses of the PVA/PEG blended films were performed to identify the components and determine the molecular attribute of the film. Figure 1 presents that the spectrum of all selected films was similar for showing O–H stretching at 3276 cm^{-1}, C–H stretching at 2939 cm^{-1}, and C–O stretching at 1091 cm^{-1}. The spectra were similar, though the components were different because the structure of PVA and PEG were close in terms of functional groups and bonding. Furthermore, the film

with PEG presented slightly broader C–O stretching due to the ether C–O in PEG, which would appear at 1275-1200 cm^{-1}. Thus, the spectra did not show any significant crosslinking between the polymers.

The SEM images of the F1, F2, and F5 films are presented in Figure 2 (a-c). The blended films were homogenous as no phase separation was presented, demonstrating the miscibility of PVA and PEG 1000 despite the PEG 1000 ratio being altered. The F1 and F2 films depicted a smooth surface film with no cracking or any undesirable characteristics. On the contrary, the F5 film, which contained the highest amount of PEG 1000, was not as smooth as others due to the presence of porosity that may have been caused by the evaporation of water adsorbed by PEG 1000. Furthermore, as demonstrated in Figure 2 (d), the FLU-film was quite smooth with little film shrinking, which might have happened upon drying. No drug particles or aggregates were seen on the film, indicating that the drug was homogenously dispersed.

Table 1 The tensile strength analysis of the PVA/PEG 1000 blended film ($^{\#}$significant difference from the F1 film, $p<0.05$), (*significant difference from the other formulation film, $p<0.05$)

Formulation	PVA (% wt)	PEG 1000 (% wt)	Stress (MPa)	Strain (%)	Young's Modulus (MPa)
F1	100	-	41.41 ± 1.10	125.85 ± 1.77	155.13 ± 15.36
F2	95	5	32.81 ± 2.59$^{\#}$	132.63 ± 2.50$^{\#}$	103.00 ± 17.06
F3	90	10	30.86 ± 3.64	130.13 ± 5.51	106.73 ± 12.42
F4	85	15	30.71 ± 1.71	134.78 ± 4.30	90.30 ± 15.52
F5	80	20	26.37 ± 1.81*	135.79 ± 7.29	79.03 ± 19.47*

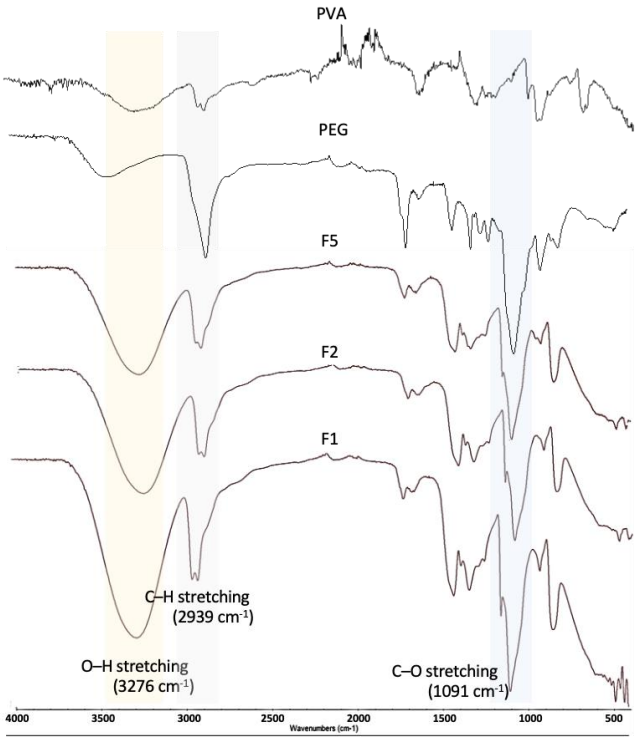

Figure 1 ATR-FTIR spectra of the PVA, PEG, and PVA/PEG films

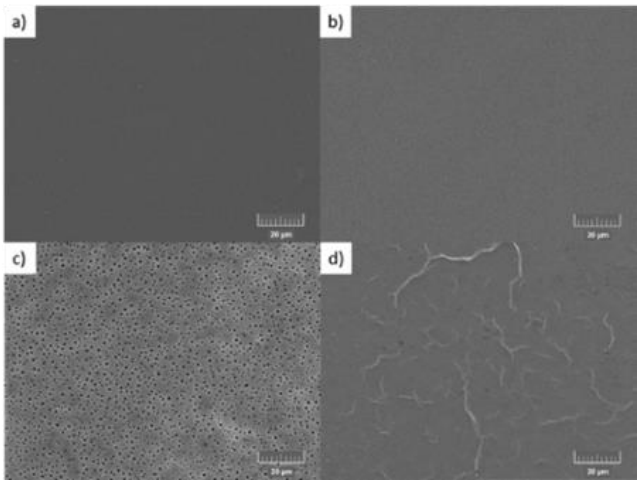

Figure 2 SEM images at 1.5k× of a) F1 (PVA:PEG 1000 = 100:0), b) F2 (PVA:PEG 1000 = 95:5), c) F5 (PVA:PEG 1000 = 80:20), and d) FLU-film (FLU-loaded into F2), each scale bar represents 20 µm

Swelling index

After soaking the film in PBS for 24 h, the % swelling of F1, F2, and F5 were 383.2±48.5, 342.5±74.5, and 353.0±85.5, respectively. This suggested that the swelling properties of all films were similar (p>0.05) regardless of the PEG 1000 content. Despite the similarity, the swelling was desirable for wound healing and was in concordance with Ngadaonye et al. (2013), which developed chitosan-poly (*N*, *N*-diethylacrylamide) IPN films for wound healing.[14] Therefore, the films presented appropriate swelling characteristics for the appointed application.

After considering several factors, including tensile strength, morphology, and swelling behavior, the F2 film was selected for further use due to its optimal tensile and morphological characteristics to be used on the skin.

Drug loading and homogeneity

FLU was added to the polymer blend to contain 0.5% w/w FLU based on the commercial FLU cream. 50 µg were integrated into the 10 mg PVA/PEG 1000 blended film. The determined quantity of FLU observed was 100.3 ± 12.0% of the stated amount. This suggested that there was no loss of FLU during the preparation process and that the drug may have been homogenously dispersed throughout the film.

Cytotoxicity

HSF cells were used to test the cytotoxicity of FLU, blank film, and FLU-film. After 24 h of exposure to the film, over 80% of the cells were still viable (Figure 3). This suggested that free FLU, blank film, and FLU-film were safe for human skin cells and that the formulated product was biocompatible. This could result from the biocompatible nature of the polymers, which have been proven safe for biological use, and the fabrication of the film did not alter their properties.

Antifungal activity

In Figure 4, the blank film did not exhibit any antifungal activity since there was no clear zone observed on the *C. albicans*-spread agar. The FLU solution showed fungistatic activity with a clear zone diameter of 30.3 ± 1.5 mm. Meanwhile, the FLU-film showed superior antifungal activity with a clear zone diameter of 35.3 ± 0.7 mm. The result suggested that once the drug was loaded into the film, the antifungal effect increased about 1.16 times compared to the free FLU (p<0.05). FLU inhibits fungal sterol synthesis, leading to aggregation of 14 alpha-methyl sterols in the fungi and finally resulting in fungistatic activity.[15] The reason that the FLU-film showed a greater

fungistatic effect may be due to the increased solubility of the drug upon preparation through the solid dispersion technique.[16] During this stage, FLU was solubilized with the polymer matrix. Subsequently, the solution was poured onto a level surface and let dry, leading to the creation of a solid film. During the process of solvent evaporation, FLU was uniformly distributed throughout the polymer network either at a molecular or particulate level, resulting in the formation of a solid dispersion. Not only does this method ensure a uniform spread of FLU across the film, but it also enhances its release pattern and ability to be absorbed by the body, hence improving the film's overall effectiveness against fungal infections.

Drug release

The drug release results in Figure 5 suggested that the FLU-film exhibits a prolonged drug release profile with approximately 70% released after 24 h, whereas the FLU solution released 100%. Furthermore, the release profiles of FLU were analyzed using different kinetic models, including zero-order, first-order, Higuchi's, and Korsmeyer-Peppas model. The linear regression coefficient (R^2) of each release profile was calculated to determine the best fit between the model and the experimental data. Based on the results presented in Table 2, it was concluded that the release of FLU through the dialysis bag membrane follows the first-order kinetic model. This suggests that the concentration gradient plays a significant role in influencing the release rate of the drug solution. The Korsmeyer-Peppas model was found to best fit with the release of FLU from the film. This model explains that the drug release from the polymeric matrix was influenced by the swelling of the polymeric matrix, which impeded the release rate of the drug. The Korsmeyer-Peppas model is often used to describe drug release from polymeric systems where diffusion through a matrix or barrier is the mechanism governing release. In summary, the study demonstrates that the FLU-film can control the release of the FLU drug, and this controlled release behavior is characterized using kinetic models that describe the release mechanism through different mediums.

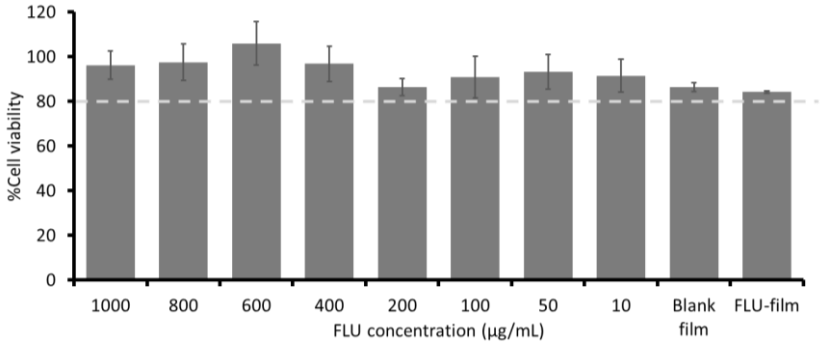

Figure 3 Cell viability (%) of FLU solution at different concentrations, blank film, and FLU-film

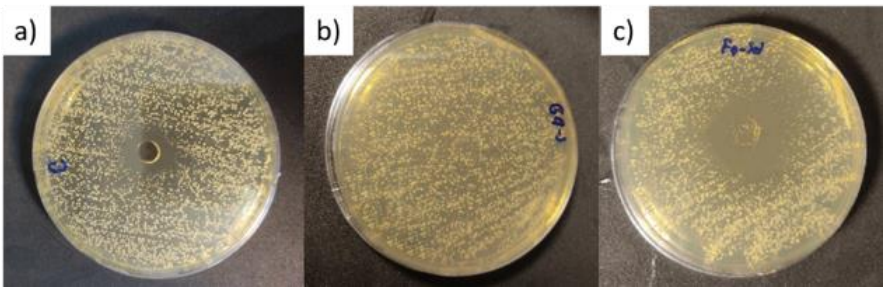

Figure 4 Antifungal activity of a) FLU solution, b) blank film, and c) FLU-film

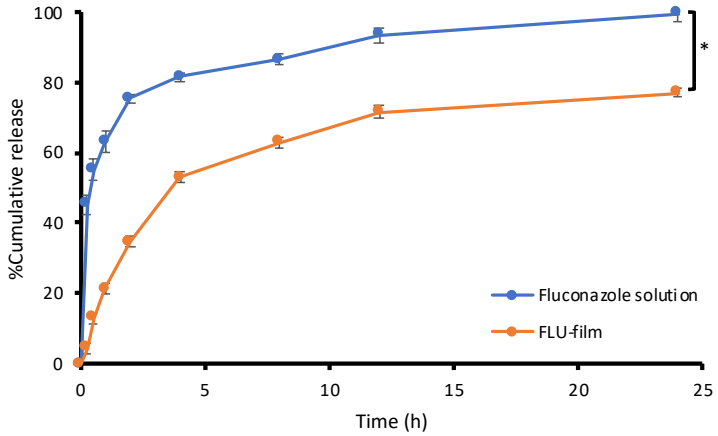

Figure 5 The release profiles of FLU-film and FLU solution (n=3)

Table 2 Drug release kinetic model represented by the R^2 of the linear release profile

Samples	Zero order	First order	Higuchi	Korsmeyer-Peppas
FLU solution	0.4634	0.9488	0.7091	0.3682
FLU-film	0.7035	0.8336	0.9118	0.9313

Conclusion

A solution casting approach was used to make PVA/PEG 1000 blended films with varied PEG 1000 percentages. As demonstrated by the tensile analysis, ATR-FTIR, SEM, and swelling index, the most suitable film for wound healing application was PVA:PEG 1000 (95:5) due to its flexibility, stretchability, swelling capability, and smooth surface. The film was prepared to contain 0.5% w/w FLU, where a homogenous drug-loaded film with no drug loss during the fabrication process was found. The components of the film and the active compound were non-toxic to the HSF cells. Notably, FLU-film outperformed a 0.5% fluconazole solution in terms of antifungal activity with the capability of controlled drug release. Finally, the FLU-film displayed good mechanical properties, biocompatibility, and fungistatic efficacy, yet further *in vivo* studies should be evaluated in the future.

Conflict of interest

There is no conflict of interest to declare.

Acknowledgments

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