Materials Science and Engineering C 73 (2017) 130–136

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Terbinafine-loaded wound dressing for chronic superficial fungal infections

Farnoush Asghari Paskiabi [a,b], Shahin Bonakdar [c], Mohammad Ali Shokrgozar [c], Mohammad Imani [d], Zahra Jahanshiri [a], Masoomeh Shams-Ghahfarokhi [e], Mehdi Razzaghi-Abyaneh [a,b,*]

[a] Department of Mycology, Pasteur Institute of Iran, Tehran 13164. Iran
[b] Microbiology Research Center, Pasteur Institute of Iran, Tehran 13164. Iran
[c] National Cell Bank Department, Pasteur Institute of Iran, Tehran 13164, Iran
[d] Department of Novel Drug Delivery Systems, Iran Polymer and Petrochemical Institute, Tehran, Iran
[e] Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form 19 November 2016
Accepted 16 December 2016
Available online 18 December 2016

Keywords:
Terbinafine hydrochloride
Dermatophytes
Wound dressing
Gelatin
Polycaprolactone
Superficial fungal infections

ABSTRACT

In spite of developing new drugs and modern formulations, the treatments of chronic fungal infections are still challenging. Fibrous wound dressings are new suggestions for the treatment of chronic superficial infections. In the present study, we formulated an antifungal agent, terbinafine hydrochloride (TFH), which is a hydrophobic drug, in wound dressings prepared by electrospun polycaprolactone, polycaprolactone/gelatin (50:50 w/w) and gelatin. To obtain more water-stable meshes, the preparations were treated by glutaraldehyde and their properties were determined before and after treatment. The morphology of fibrous meshes was observed by scanning electron microscopy. Drug loading efficiency and release rate were measured by high performance liquid chromatography (HPLC) and the release rate was monitored for 144 h. Antifungal tests were performed on *Trichophyton mentagrophytes*, *Aspergillus fumigatus* and *Candida albicans* cultured on Muller-Hinton agar. The toxicity of the meshes was measured after 24 h and 14 days by MTT assay. Terbinafine loading of polycaprolactone/gelatin (50:50) was 100% and it released the highest amount of TFH too. In antifungal tests, all samples were able to hinder*T. mentagrophytes* and *A. fumigatus* but not *C. albicans* growth among them, polycaprolactone fibers made the largest inhibition zone. In MTT assay, none of prepared samples showed toxicity against L929 cells. Teken together, the prepared TFH-loaded PCL/gelatin electrospun meshes were able to release TFH slowly and in a steady state in time. With respect to no obvious cytotoxicity in MTT assay and stong antifungal activity toward *T. mentagrophytes in vitro*, these TFH-based meshes could be considered as potential candidates in clinical application as wound dressing for treatment of chronic dermatophytosis.

1. Introduction

The responsible microorganisms for human fungal infections are spread throughout the world [1]. Despite develoing new drugs and more effective formulations of antifungals in the last decade, the rate of people who affected by fungal infections is still considerable. For example between 2006 and 2009, 39% of patients referred to Mycology Department of the Pasteur Institute of Iran were positive for *Candida*, dermatophytes or opportunistic saprophytes of which 54% suffered from chronic fungal dermatophytosis [2].

Recently, topical drugs have been introduced as new medications to combat different kinds of fungi. The treatment of chronic nail dermatophytic infection is very difficult because of little and late drug penetration from the ointment into epidermis.

On the other hand, oral antifungals may cause severe liver toxicity, rare skin problems such as Stevens-Johnson and probable drug-drug interaction through cytochrome P-450 metabolism. Moreover, oral antifungals are not allowed for children [1].

Nowadays, more effective formulations for drug delivery into fungal wounds are required. A wound dressing can be made of electrospun polymers which possess a drug releasing system. In comparison with traditional mats, more amounts of gases can pass through electrospun wound dressings and they protect wounds from water lost and infection [3]. These structures can help homeostasis phase because of their wide surface area and small holes [4]. Their water absorption is calculated much higher than traditional films [5]. Because of this property, the nanofibrous dressings made of hydrophilic polymers absorb wound exudates very effectively [4]. According to Vasita et al. (2006) wound dressings made from polyurethane are very penetrable to oxygen and

* Corresponding author at: Department of Mycology, Pasteur Institute of Iran, Tehran 13164, Iran.
E-mail addresses: mrab442@yahoo.com, mrab442@pasteur.ac.ir (M. Razzaghi-Abyaneh).

http://dx.doi.org/10.1016/j.msec.2016.12.078
0928-4931/

control water evaporation successfully. These nanofibers allow the exudates come out the wound while prevent the wound drying [6]. One method to fabricate fibrous wound dressing is electrospinning [7–9].

In electrospinning, a polymer(s) solved in an appropriate solvent is injected through a metal needle toward a conductive surface. A high voltage supplier applies voltage to the needle as well as the polymer solution's droplet on top of the needle. The charge overcomes the surface tension of the droplet and after making Taylor cone, the polymer solution's droplet flies toward the front conductive surface. During the flight, the solvent evaporates and the polymer deposits on the surface in fibrous form. These fibrous meshes are being used as wound dressings [4,10–12].

Nanofibers can be used in drug delivery systems. Incorporating of therapeutic compounds like antiseptics, antibiotics and growth factors into electrospun fibrous mats is simply possible [4]. The release mechanisms of rifampin, doxorubicin hydrochloride and paclitaxil have been previously studied [13]. They were incorporated in poly-L-lactic acid (PLLA) electrospun fibers. The release of rifampin did not happen before using proteinase K. The drug did not permeate or diffuse from fibers into the environment and the only way of release was polymer degradation. They found doxorubicin hydrochloride on the surface of nanofibers, while paclitaxil did not leave the nanofibers. The hydrophilic doxorubicin hydrochloride had limited solubility in the electrospinning solution and because of phase separation between drug and PLLA during solvent evaporation in electrospinning, moved onto the fiber surface. Both rifampin and paclitaxil were soluble in the PLLA and the solvent and did not come out from the fiber during electrospinning process [14].

The release rate of itraconazole and ketanserin from a hydrophobic polymer is also examined. Both itraconazole and ketanserin, which are poorly water-soluble drugs, released from hydrophobic polymeric fibers through diffusion mechanism, while ketanserin initial release was faster than itraconazole. Higher solubility and diffusivity of ketanserin in the polymer was suggested as reason of this difference [15].

The nanofibrous systems provide wide surface area and high porosity which facilitate drug penetration and modify water solubility of drugs. These systems can substitute systemic or oral formulation of drugs, decrease drug dose and prevent drug side effects [16].

Terbinafine hydrochloride is a topical or oral active allylamine. Because of its versatile antifungal activity and low required dose, it is an appropriate candidate for transdermal drug delivery systems (TDDS). Terbinafine hydrochloride can fight against a wild range of dermatophytes, dimorphic fungi, dematiaceous fungi and yeasts [17]. Chemical formula of terbinafine hydrochloride is (*E*)-N-(6,6-dimethyl-2-hepten-4-ynyl)-Nmethyl-1-naphthalenemethanamine hydrochloride. It inactivates fungal squalene epoxidase [18], so hinders fungal ergosterol biosynthesis [17]. Terbinafine hydrochloride is a fungicidal that shortens treatment period [19] and is official in United States, European and British Pharmacopoeia [20].

In the present study, electrospun fibers containing terbinafine hydrochloride as an antifungal wound dressing were fabricated. The release rate of terbinafine hydrochloride (as a hydrophobic drug) was regulated by using a blend of hydrophobic and hydrophilic polymers in the wound dressing structure. Polycaprolactone (PCL) is a hydrophobic synthetic polymer which has been electrospun successfully before [21,22] and gelatin is a hydrophilic natural polymer which is an appropriate candidate in medical applications [23,24]. The electrospun fibers were made from PCL, PCL/gelatin and gelatin and their drug release rate, antifungal effects and toxicity were determined.

2. Materials and methods

2.1. Materials

Polycaprolactone (Mn ≈ 80,000) was purchased from Sigma, hexafluoroisopropanol (HFIP) from Trademax Pharmaceutical & Chemicals Co., Ltd. (China) and terbinafine hydrochloride from Dr. Reddy's Laboratories Limited. Gelatin (for microbiology), methanol, triethylamine (TEA) and glutaraldehyde 25% were purchased from Merck (Germany). *Trichophyton mentagrophytes* (PTCC 5054), *Aspergillus fumigatus* (Af293) and *Candida albicans* (ATCC 10231) were supplied by Pathogenic Fungi Culture Collection (PFCC), Pasteur Institute of Iran and L929 cell line was obtained from National Cell Bank of Iran (NCBI). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma-Aldrich, USA and Gibco/Thermo Fisher Scientific, USA, respectively.

2.2. Wound dressing preparation

The meshes were prepared in 3 steps: solution preparation, electrospinning and crosslinking.

2.2.1. Electrospinning solutions

Polymer solutions (10%) of polycaprolactone, gelatin and polycaprolactone and gelatin blend (50:50) were prepared in HFIP. For drug-loaded samples, terbinafine hydrochloride (TFH) was added equal to 5% of the polymer(s) weight.

2.2.2. Electrospinning process

A syringe with 27-G needle was used for polymer solution injection. The needle tip distance from the drum was 15 cm. The solutions were injected with 0.2 ml/h flow rate while a high-voltage power supply was applying 25–40 kV voltages to the needle. Electrospun fibers were collected on a 25 × 10 cm^2 alumonium foil which was wrapped around the rotating drum. The whole process was done at 25 °C. Details of electrospinning conditions were shown in Tables S1, S2 and S3.

2.2.3. Crosslinking

A solution of GTA in deionized water (4%) was used for vapor cross linking. PCL, PCL/gelatin and gelatin electrospun fibers (1 × 1 cm^2 pieces) were put in inside a desiccator without any direct contact with the GTA for 24 h [25].

2.3. Scanning electron microscopy (SEM)

Electrospun fibers were sputter coated with gold and visualized by scanning electron microscope with accelerating voltage of 20.00 kV (VEGA//TESCAN, Czech Republic).

2.4. Drug release studies

2.4.1. Drug loading measurement

The amount of 1 ml HFIP was added to each sample and stirred (500 rpm, 30 min) to solve the polymers. Afterwards, 3 ml methanol was added and stirred for 30 min at 500 rpm. Next, the solution was centrifuged, filtered and analyzed for terbinafine hydrochloride loading by HPLC (KNAUER, Germany). Analysis performed at 220 nm with a C18 reversed-phase column of 15 cm × 4.6 mm i.d. and 5 μm dimensions and a TSK GUARDGEL, ODS-120A (19005) pre-column. The mobile phase was an equal proportion of A (0.012 M triethylamine and 0.02 M orthophosphoric acid) and B (acetonitirile) [26]. Flow rate was set at 1 ml/min. The drug loading percent was obtained via the following formula:

$$\text{Drug loading } (\%) = (\text{measured drug mass}/\text{calculated drug mass}) \times 100$$

2.4.2. Drug release

Electrospun meshes containing terbinafine hydrochloride (GTA treated or not) in approximate dimensions of 25 × 10 cm^2 of each