THE EFFECT OF PCL/PLA BLEND NANOFIBERS WITH CLOTRIMAZOLE ON CANDIDA ALBICANS GROWTH

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Abstract

Polycaprolactone (PCL) and polyl(actic acid) (PLA) are biocompatible and biodegradable polymers widely used in biomedical applications. This study explores the fabrication and antifungal efficacy of electrospun nanofiber blends of PCL/PLA incorporated with clotrimazole, an imidazole-based antifungal agent, targeting *Candida albicans*. Nanofibers were produced by needless electrospinning with different clotrimazole concentrations. Morphological analysis using scanning electron microscopy confirmed smooth fiber formation with diameters affected by the clotrimazole content. In vitro antifungal assays were conducted using *Candida albicans*, assessing fungal growth inhibition through zone of inhibition tests and the effect on growth curve by nanofiber eluate. Results demonstrated that clotrimazole-loaded PCL/PLA nanofibers significantly inhibited *Candida albicans* growth compared to unloaded controls, with the most pronounced effect observed in nanofiber mats containing the highest dose of clotrimazole (~ 40 µg/ mg). Cytotoxicity assay confirmed biocompatibility of the nanofiber mats to human vaginal keratinocytes. This study highlights the potential of clotrimazole-loaded PCL/PLA nanofibers as a promising platform for localized antifungal therapy, particularly in treating vaginal candidiasis.

Keywords: Nanofibers, polycaprolactone, poly(lactic acid), antifungal activity, *Candida albicans*

1. INTRODUCTION

Polycaprolactone (PCL) and polylactic acid (PLA) are widely recognized biodegradable and biocompatible polymers with extensive applications in biomedical engineering, particularly in drug delivery and tissue scaffolding [1,2]. Their favorable mechanical properties, tunable degradation rates, and compatibility with electrospinning techniques make them ideal candidates for production of nanofiber-based systems. Electrospun nanofibers offer high surface area-to-volume ratios and porosity, which are advantageous for controlled drug release and cellular interactions. Blending PCL and PLA allows for the optimization of fiber characteristics, balancing flexibility and degradation kinetics to suit specific therapeutic needs, including potential vaginal drug delivery [3].

Clotrimazole, an imidazole-based antifungal agent, is commonly used to treat infections caused by *Candida albicans*, a prevalent opportunistic fungal pathogen responsible for mucosal and systemic candidiasis. Conventional clotrimazole formulations often suffer from limited retention at the site of infection and suboptimal

release profiles [4]. Incorporating clotrimazole into electrospun nanofibers presents a promising strategy for localized, sustained antifungal therapy. Such systems may enhance drug stability, prolong release, and improve therapeutic efficacy while minimizing systemic side effects [5].

This study focuses on the fabrication of PCL/PLA nanofiber mats loaded with varying concentrations of clotrimazole using a needleless electrospinning method. The morphological properties of the fibers were characterized using scanning electron microscopy, and their antifungal activity was evaluated through in vitro assays against *Candida albicans*. Zone of inhibition tests and growth curve analyses were employed to assess the extent of fungal suppression by clotrimazole-loaded nanofibers compared to unloaded controls. Furthermore, the biocompatibility of the nanofiber mats was assessed using metabolic cytotoxicity assays on human vaginal keratinocytes to ensure safety for potential mucosal application.

2. MATERIALS AND METHODS

2.1 Nanofibrous sheets preparation and morphological analysis

The polymers used for nanofibers preparation were biodegradable polyesters poly(lactic) acid (PLA, Corbion, Purac Biochem bv, NL) and poly-ε-caprolactone (PCL, Sigma-Aldrich, Mw 80,000). Antimycotic was Clotrimazole (ThermoFisher), dissolved in absolute ethanol (Penta). The ratio of the polymers in spinning solution was PCL: PLA 75:25, the concentration of Clotrimazole in solution was 0.1 mg/ml, 1 mg/ml and 10 mg/ml. Nanofibrous sheets were prepared from the chloroform-based solvent system with DC needleless electrospinning device Nanospider™ (NS Line 1WS500U, Elmarco), set to production speed 15 mm/min. Morphology of the nanofibrous sheets was evaluated using scanning electron microscopy (SEM) with a (Tescan Vega 3 SEM). The fiber diameters were measured using built-in software and expressed as histograms of diameter distribution and mean ± standard deviation.

2.2 Evaluation of antifungal activity by disc diffusion method

Candida albicans strain (ATCC 24433, CCM 8320) was purchased at Czech Collection of Microorganisms (MUNI, CZ) as a refence strain for antifungal susceptibility testing. Experiments were conducted from overnight culture in Yeast Nitrogen Base with amino acids (YNB) (Sigma Aldrich). To test antifungal activity of the materials in vitro, 1 ml of yeast suspension (turbidity 0,5 McFarland) of *Candida albicans* was seeded on Sabouraud agar plates (4 % glucose, Roth) and nanofibrous discs (10 mm of diameter) were placed on the agar in triplicates and incubated for 24 hours at 30 °C. After the exposition period, size of diffusion zone inhibiting bacterial growth around samples was measured and photo documented.

2.3 Growth curve of Candida albicans in eluate from nanofibrous sheets

The materials were incubated in YNB media, 10 mm disk of each nanofiber material in 2 ml of YNB for 24 hours in 37 °C to prepare the eluate of each material. Yeast suspension (0,5 McFarland) was then mixed 1:1 with each eluate in 96-well mictrotiter plate in triplicates and incubated with gently agitation in 30 °C. The growth of the yeast was assessed by measuring optical density at 550 nm (OD 550) at 1-hour intervals for 24 hours, with three measurements per each well.

2.4 *In vitro* cytotoxicity of nanofibrous sheets in direct contact with vaginal keratinocytes

Vaginal keratinocytes VK2/E6E7 obtained from ATCC (CRL-2616) were used for cytotoxicity evaluation experiment. The cell line was maintained in Keratinocyte serum-free medium (Invitrogen), supplemented with bovine pituitary extract (50 g/ml) and epidermal growth factor (0.1 ng/ml). Cytotoxicity was assessed by MTT assay. The keratinocytes were seeded 20,000 cells per well in 24-well plate a day before the experiment. At 24 hours after seeding, each material was added into the well in triplicate (60 mm diameter discs) with fresh

media and after 24 hours, the viability of cells was measured using MTT assay. Samples with viability exceeding 70 % of the cell control are considered biocompatible.

3. RESULTS AND DISCUSSION

3.1 Fabrication of nanofibrous materials and its morphology

In this work, three types of biodegradable nanofibrous sheet materials (NFs) were produced, containing PCL:PLA 75:25 mixture of polymers and three concentrations of Clotrimazole (**Table 1**).

Table 1 Volumetric preparation of spinning solution of three NFs and its mass ratio of Clotrimazole to NFs

Volumetric spinning solution	Mass ratio of Clotrimazole to NFs
PCL:PLA + 0.1 mg/ml Clotrimazole	0.3898 μg Clotrimazole/mg NFs
PCL:PLA + 1 mg/ml Clotrimazole	3.95 μg Clotrimazole/mg NFs
PCL:PLA + 10mg/ml Clotrimazole	36.736 μg Clotrimazole/mg NFs

Analysis of morphology of the materials showed fibrous structure with randomly oriented nanofibers. Mean of nanofiber diameter increased with increasing concentration of incorporated Clotrimazole, from 445.02 nm in PCL:PLA + 0.1 mgl/ml Clotrimazole to 535.32 nm in PCL:PLA + 10 mg/ml Clotrimazole (**Figure 1**).

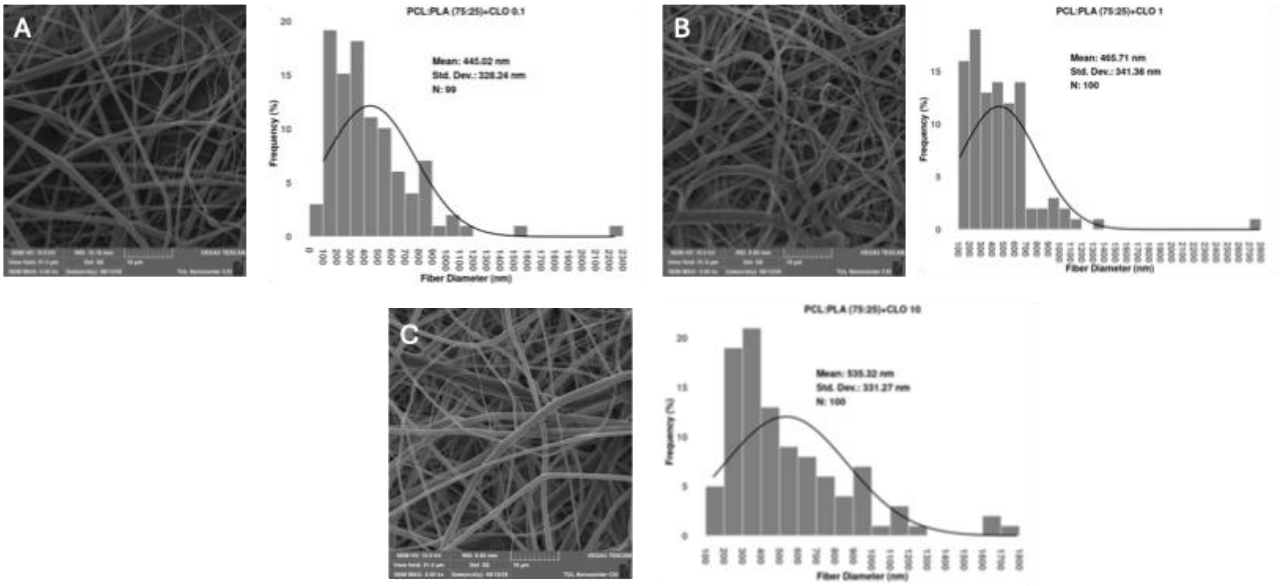

Figure 1 Morphology of nanofibrous sheets using SEM with histograms of relative frequency of the fiber diameters. A – PCL:PLA + 0.1 Clotrimazole, B – PCL:PLA + 1 Clotrimazole, C – PCL:PLA + 10 Clotrimazole. SEM magnification 5,000x (bar = 10 μm)

3.2 Evaluation of antifungal activity by disc diffusion method

All the materials exhibited a distinct inhibition zone after 24 hours incubation, with the diameter of the zone increasing with the increasing concentration of incorporated Clotrimazole (**Figure 2**). The measurements of inhibition zones are shown in **Table 2.** This clearly shows the ability of the antimycotic drug to diffuse effectively from the nanofibers and act as a growth inhibitor and mycotoxic agent depending on its concentration in the material.

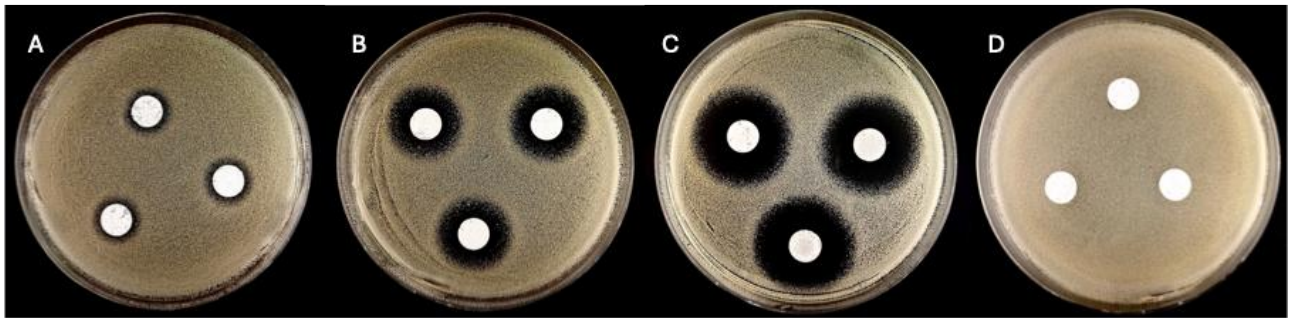

Figure 2 Inhibition zones of materials in contact with Candida albicans on Sabouraud agar.
A – PCL:PLA + 0.1 Clotrimazole, B – PCL:PLA + 1 Clotrimazole, C – PCL:PLA + 10 Clotrimazole,
D – PCL:PLA nanofibers without Clotrimazole.

Table 2 Inhibition zone for Candida albicans and nanofibrous materials with Clotrimazole, 24 h incubation

	PCL:PLA + 0.1 CLO	PCL:PLA + 1 CLO	PCL:PLA + 10 CLO
Inhibition zone (mm)	14,556	22,778	30,334
Standard deviation	0,769	0,384	0,577

3.3 *In vitro* cytotoxicity of nanofibrous sheets in direct contact with vaginal keratinocytes

The evaluation of viability of vaginal keratinocytes in direct contact with Clotrimazole-incorporated nanofibers (**Figure 3**) shows biocompatibility of the materials with lower concentration of antimycotics, where both PCL:PLA + 0.1 Clotrimazole and PCL:PLA + 1 Clotrimazole show that the cells are viable above 70 % after 24 hours of direct exposure to the materials. On the other hand, the nanofibrous sheet with the highest concentration (PCL:PLA + 10 Clotrimazole) appears to inhibit the growth and metabolic activity of the vaginal keratinocytes, as the viability is under the 70 % threshold.

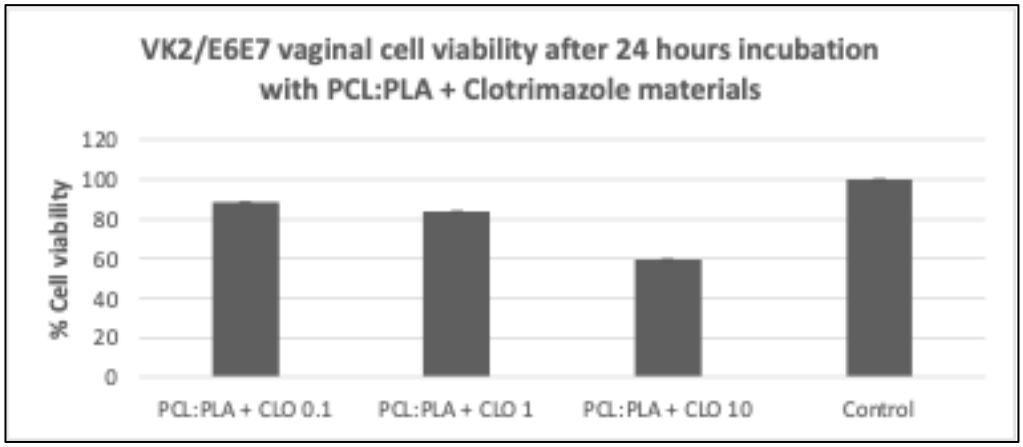

Figure 3 Biocompatibility evaluation in vitro. Cells: VK2/E6E7 vaginal keratinocytes, materials 60 mm discs, exposure 24 hours

3.4 Growth curve of Candida albicans in eluate from nanofibrous sheets

Incubation of Candida albicans suspension with the eluate from Clotrimazole nanofibers resulted in different shape of growth curve. Even though the yeast cells were able to overgrow the effect of Clotrimazole in the media and reach similar optical density after 24 hours, the initial effect on the length of lag phase and steepness of the exponential phase is dependent on Clotrimazole concentration, as evident from **Figure 4.** The eluate from material with the lowest concentration of Clotrimazole did not have measurable effect on the growth of

Candida albicans cells in contrast to results from disc diffusion test. This is likely due to the difference in effect of direct contact with the material (in disc diffusion) and effect of eluate, which does not exhibit the same impact on growth of yeast cells.

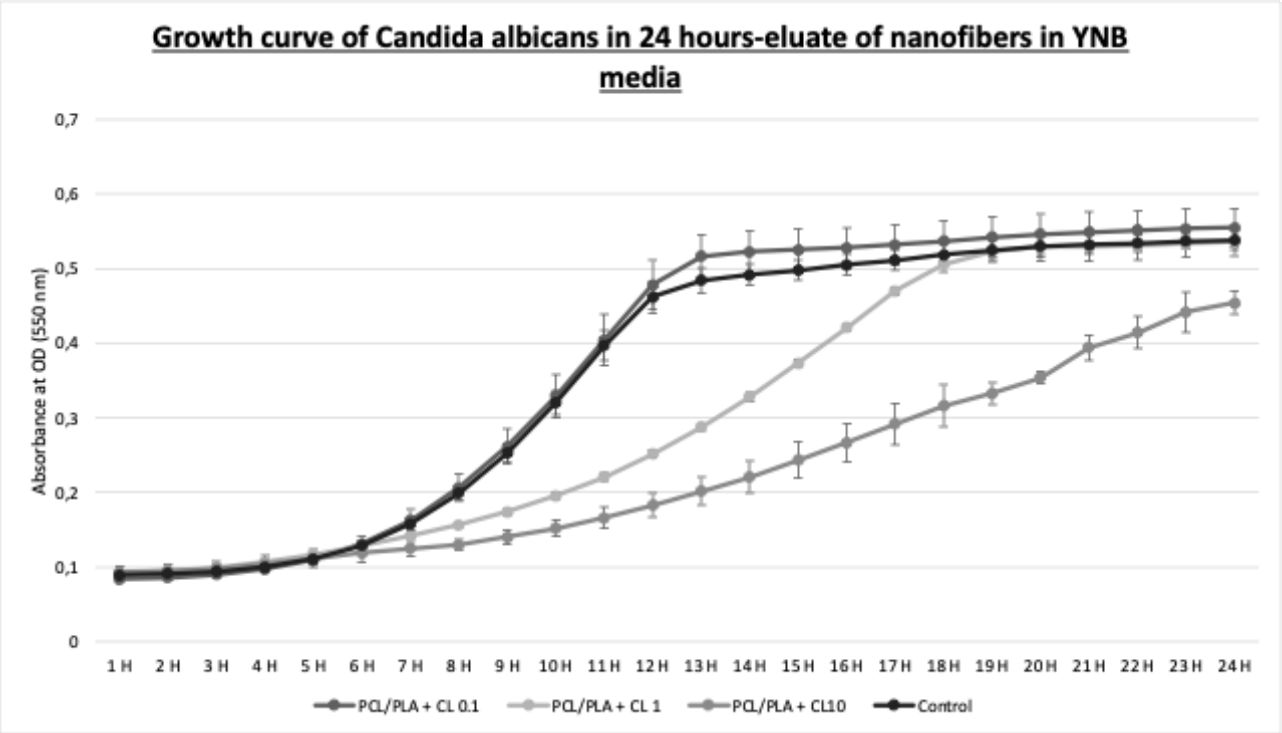

Figure 4 Growth curve of Candida albicans in 24 hours-eluate of nanofibers in YNB media, 30°C

Collectively, these findings suggest that the 1 mg/mL clotrimazole-loaded nanofibers offer the most promising balance of antifungal activity and cytocompatibility, making them the optimal candidate for further research.

4. CONCLUSION

The objective of this study was to evaluate the effect of PCL:PLA nanofibrous material with incorporated antimycotic drug Clotrimazole on the growth of Candida albicans and its biocompatibility with vaginal keratinocytes. The concentration of Clotrimazole affected the diameter of nanofibers, with the increasing diameter correlating with increasing concentration of the drug. Clotrimazole was effective in growth inhibition in disc diffusion test as well as affected the growth kinetics when used as a eluate. The cytotoxicity test shows that the highest concentration of Clotrimazole has cytotoxic effect on cells. These findings indicate, that the concentration of 3.95 μg Clotrimazole/mg NFs is both effective against the pathogen and at the same time biocompatible with the vaginal epithelia, making it a valid candidate for future testing of drug delivery for the treatment of vaginal candidiasis.

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