Drug-loaded Electrospun Nanofiber for Vulvovaginal Candidiasis: A Systematic Review

**Running title- Nanofiber for VVC.**

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Abstract

**Purpose:** This study decodes the role of electrospun nanofibers in the treatment of VVC.

**Result:** 7 out of 8 included studies were basic research with in vitro data and one of them is showing in vivo data. All of the included articles showed the mucoadhesive nature of nanofibers with good tensile strength, good drug absorption with improved bioavailability, and sustained drug release with better therapeutic activity than other formulations.

**Conclusion:** Nanofibers found as a potential carrier that overcomes the constraints of the conventional formulations for the delivery of drugs in the vagina and warranted an effective treatment for VVC.

**Keywords:** Nanofibers; Electrospun; Vulvovaginal Candidiasis; Drug delivery; Polymer.

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1. INTRODUCTION

Vulvovaginal Candidiasis (VVC) is the most prevalent health burden among women of reproductive age [1]. Globally, approximately 138 million women are affected by VVC annually, and 372 million are affected over their lifetime [2]. VVC is mainly caused by Candida yeast species that infect the vagina, signalized by pruritus, hyperemia, burning sensation, dyspareunia, erythema, and curd-like white discharge [3]. Approximately half of women once in their life are affected by VVC once in their lives, with one or two episodes [4-5]. More than 3/4th of the cases of VVC are caused by Candida albicans (C.albicans), and the remaining cases are due to other Candida species such as C.krusei, C.glabrata, C.tropicalis, and C.parapsilosis [6]. Vaginal douching, frequent use of antibiotics, uncontrolled diabetes, improper sexual activity, tight-fitting underwear, dermatitis, imbalance in vaginal micro-flora, higher estrogen levels, contraceptives, pregnancy, use of antiseptics, immunosuppression, and hormone replacement therapy induce VVC infection [7-8]. Hormonal activity, presence of microbiota and enzymes, change in pH, excessive secretion of vaginal fluid, and thickness of the vaginal tissue layer that catalyzes with age, which alters the absorption and bioavailability of the drug, make the vagina an intricate route for drug delivery [9-10]. Drug absorption and stability may also be affected by the solubility, dissociation constant, molecular size, and dissolution of the drug in the vaginal content [11,12]. Conventional formulations such as gels, creams, solutions, and foams have poor retention and distribution of drugs in the vagina [13-14]. Therefore, nanotechnology has received considerable attention for appropriate localized drug delivery via the vaginal route [15-16]. In the last two decades, nanotechnology has emerged as a revolutionary step in the pharmaceuticals industry to improve the uniform delivery of drugs, increase efficacy, decrease side effects and the frequency of doses or drug regimens, prolong drug release, and improve therapeutic effects [17-18]. Nanofibers have emerged as novel nanotech applications designed for drug delivery, tissue healing, wound dressing, and implants after surgery with numerous advantages [19-20]. Elongated filamented nanofibers with multiple structures such as ribbon, core-shell, single and multi-layers, multichannel, porous, necklace, and web-like structures that manifest unique features with a rough size range of less than 500 nanometers (nm) used for local applications as well as systemic delivery [21-22]. Fabrication of nanofibers through the electrospinning technique has outstretched at a height in the field of nanotechnology, which involves rapid evaporation and sudden extension of active agent-loaded polymeric solutions leading to the conversion of a crystalline form of drugs into an amorphous form to an increase in solubility, resulting in
enhanced bioavailability [23-24]. Drug-loaded nanofibers show efficacy that is related to the amount of drug-loaded and elevated drug-loading capacity in nanofibers owing to the large surface area and uncomplicated fabrication method [25-26]. Fluctuations in drug loading can be affected by the type of electrospinning method used for fabrication, polymer types and their density, solvent used, and technique used for drug loading [27].

1.1 Advantages and disadvantages of nanofibers over conventional formulations

Nanofibers have an exceptionally high surface area with better mucoadhesion that allows efficient contact with the targeted site and facilitates absorption. Nanofibers elevate bioavailability with efficacy and control the release of active ingredients which lowers the side effects. The controlled release maintains the therapeutic drug levels, reduces frequent dosing, and improves patient compliance [28-29]. The flexible nature of nanofiber promotes smooth application at the infection site and also improves the stability of drugs by increasing their shelf life. Satisfactory mechanical strength maintains the stability and integrity of the nanofiber and opts better tool to overcome the constraints of conventional formulations in VVC management.

The material used to fabricate nanofiber might not always be biocompatible or safe for prolonged exposure [30]. The degradation rate of nanofibers depends on the types of material and the environmental condition. The electrospinning technique requires significant parameters to fabricate nanofibers. Nanofibers might undergo structural modification or change in properties over time, affecting their drug-release pattern and overall effectiveness [31-32]. Personalized options, long-term safety, and real-world effectiveness need to be carefully evaluated. Considering all the pros and cons of nanofibers, the current study aimed to systematically update the literature that focused specifically on the drug-loaded nanofibers preferred in the treatment of VVC.

2. METHODS:

The study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines.

2.1 Search Strategy

The search was performed using PubMed/Medline, Google Scholar, and Science Direct till October 31, 2022. Search terms were developed using the following search strings: (nanofiber) OR (nanofibrous)) AND (electrospinning)) AND (drug loaded) AND (vulvovaginal
candidiasis) OR (VVC). The study was confined to articles issued between 2000-2022 in English only. References to the included articles were also retrieved to boost the search terms.

2.2 Inclusion and exclusion criteria

Two reviewers (AV and AKS) individually screened all titles and abstracts, followed by a full-text screening based on the inclusion criteria. Any conflict over inclusion was resolved by the third (TR) and fourth (MA) reviewers. The final decision was made by the fifth and Sixth reviewer (SP) and (KK) after the discussion. The criteria for inclusion were as follows: Original research articles on nanofibers fabricated by the electrospinning method, which is used specifically for VVC and published only in the English language. Reviews, editorials, book chapters, conferences, abstracts, editorials, and correspondences were excluded. The method used to fabricate nanofibers other than electrospinning was also excluded. The nanofibers used to treat other vaginal issues were similarly excluded.

2.3 Data collection and extraction

The articles were sorted by two reviewers (AV and AKS) from different databases and duplicates were removed. The retrieved articles were screened twice consecutively. Irrelevant articles were excluded and the remaining articles were analyzed based on the inclusion criteria through their abstracts and full-text screening. The data were extracted and are presented in Table 01 with subheadings such as drug-loaded, polymer used, diameter of nanofibers, types of studies involved, conclusions, and references.

3. RESULTS

3.1 Studies result

A total of 3960 hits were identified, of which 792 were from PubMed, 438 from Google Scholar, and 2730 from Science Direct. Out of the 3960 articles, only 26 articles were selected for secondary screening and only 8 articles were considered on the basis of inclusion criteria. Two studies were conducted in India, one in Brazil, two in Turkey, and three in Iran. The screening procedure for the selection of the study is presented in Figure 01 as per the PRISMA guidelines.

3.2 Studies characteristics

All relevant articles were thoroughly studied and are presented in Table 01. These studies focused on the nanofibers produced by the electrospinning method and were used only for the
treatment of VVC caused mainly by Candida albicans. Extracted information from basic research and preclinical studies ascertained that VVC can be easily treated with various drugs loaded in nanofibers which can increase the residence time of the drug and also manifest a controlled release drug pattern with efficient localized activity with no vaginal irritation and no systemic cytotoxicity. The application of polymers can modulate the nature of drug-loaded nanofibers, with respect to their compatibility and feasibility.

3.3 Nanofibers and Electrospinning method

Nanofibers can be fabricated by self-assembly, phase separation, extraction, drawing, template synthesis, melt-blowing, electrospinning, and 3D printing methods. Electrospinning is the simplest and most feasible method for the delivery of drugs, using electrostatic forces as a driving force in the fabrication of nanofibers. Different parts of the electrospinning setup include (a) polymeric solution (b) syringe (c) metallic needle or spinneret (d) syringe pump (e) voltage output device and (f) collector plate. A polymeric solution or melted polymer is forced by a syringe pump through the spinneret by applying a high voltage, resulting in the generation of charge in the polymeric solution. The charge movement causes the stretching of a spherical drop (also called a pendant drop) at the needle tip formed by surface tension. When electrostatic repulsion of charged solution overcomes the surface tension, a cone-like structure forms the so-called “Taylor cone”. Furthermore, the Taylor cone elongates into a thin fiber and extends towards the collector plate with evaporation of the solvent. Fibers can be collected on aluminum foil, glass slides, metallic plates, or rotating drums. The design of the electrospinning setup and nanofiber image obtained using a scanning electron microscope are shown in Figure 02. The selection of polymers and drugs should be biocompatible, biodegradable, non-toxic, patient-compliant, and economical for formulating nanofibers for VVC infection.

4. DISCUSSION

As per the literature survey, nanofibers’ application for VVC treatment has increased in recent years. Despite the presence of different vaginal barriers, drug delivery is a major issue that relies on conventional formulations. Therefore, many researchers have developed drug-loaded nanocarriers to overcome the drawbacks of conventional formulations. Nanofibers are novel nanotechnology-based drug carriers that provide efficient drug delivery at targeted sites [33-34]. Different studies have been performed to support the use of nanofibers in VVC. Souza et al. examined amphotericin B-loaded poly (lactic-co-glycolic acid) (PLGA) nanofibers by electrospinning, which provided localized delivery of drugs to the vagina and revealed a
controlled release of the drug for eight consecutive days. In vitro and in vivo models have been used to determine the effectiveness and potential value of antifungal activity against Candida species in the treatment of VVC. Sharma et al. prepared uniform, bead-free, fluconazole-loaded polyvinyl alcohol (PVA) polymeric nanofibers with a diameter range of 150nm-180nm through an electrospinning method. In vitro tests indicate a sustained action of 6 h and a better antifungal activity against Candida species in comparison to the plain drug. Essential oils are currently used in various formulations, owing to their satisfactory therapeutic value. Semnani et al. fabricated eugenol-loaded polyacrylonitrile (PAN) nanofibers and observed that an increase in the eugenol ratio led to an increase in the diameter of the nanofibers, and drug release was observed for 150 h by electrospinning. Najmeh et al. used polymers such as PVA, Sodium alginate, and dextran to prepare a film and nanofiber loaded with clotrimazole. Important differences between the films and nanofibers were in terms of their morphology, Young’s modulus, mucoadhesive properties, and antifungal activity. Nanofibrous mats showed better adhesion to the vaginal mucosa and higher antifungal activity than the film. A study was conducted by Fatmanur et al. in which benzydamine nanoparticles were incorporated into electrospun nanofibers and a gel with free benzydamine was prepared with better adhesive properties and a controlled drug release with greater drug penetration in the vaginal tissues. Therefore, benzydamine nanoparticles loaded in nanofibers were chosen as a better option than benzydamine gel formulation for the treatment of vaginal infections. Pursottom et al. formulated a eucalyptol/β-cyclodextrin inclusion complex to gellan/polyvinyl alcohol nanofibers (EPNF) by electrospinning that showed a prolonged release with 70% of inhibition of Candida albicans and C. glabrata biofilms. Mehran et al. prepared electrospun polycaprolactone (PCL)/polyvinylpyrrolidone (PVP) polymeric nanofibers loaded with ketoconazole and fluconazole. Using the disk diffusion method against C. albicans, ketoconazole nanofibers showed better antifungal activity than fluconazole-loaded nanofibers. Imren et al. compared polymeric nanofibers of polyurethane/polyvinylpyrrolidone loaded with the drug and polyurethane/polyvinylpyrrolidone/silk loaded with the drug. Nanofibers with silk as a polymer blend or as a coating material showed prolonged or sustained action against C. albicans infection in comparison to nanofibers without silk. As the outcome of an increase in the research on nanofibers, it can be expected that nanofibers can become a basic part of nanotechnology for the delivery of drugs in the case of VVC in the coming future. The current systematic study has some constraints. The important related articles were searched on limited databases. Only articles in the English language were taken into account. Quality assessment and publication bias were not considered for this study since the included studies
are basic research with in vitro data and also pre-clinical studies containing in vivo data. There is a dearth of clinical evidence for the use of nanofibers in VVC patients. Therefore, this review is restricted up to preclinical stage. Although in vitro and in vivo data of relevant included studies demonstrate that nanofibers are one of the best alternatives for VVC treatment as they provide prolonged residence time, better mucoadhesion, and sustained or controlled drug release that enhances absorption as well as improves bioavailability.

5. CLINICAL PERSPECTIVE OF NANOFIBERS

The ability of nanofiber to tune the characteristics of drug delivery, encapsulate a wide variety of therapeutic agents, and adhere to vaginal mucosa to target the drug delivery at the site of infection with prolonged therapeutic effect and reduced systemic side effects makes nanofiber an enable candidate to face current challenges in the treatment of VVC. Based on the promising outcomes of basic research and preclinical data, electrospun nanofibers necessitate clinical trials to showcase clinical applications. The current electrospinning technique must be amplified to boost the production capabilities for commercial access. The safety and efficacy of nanofibers must be assessed by conducting real-world studies. Though having enormous potential, electrospun nanofibers have yet to be approved as commercial advanced dosage forms. If nanofibers are augmented to the clinical level it can be a game changer in female reproductive health.

6. CONCLUSION

This review systematically updates the published articles and provides important information with evidence that drug-loaded nanofibers are potential carriers to combat VVC. Various drugs have been used for targeted drug delivery through these fibers. In vivo and in vitro tests performed in different studies have shown that nanofibers can be considered a new system for the treatment of vaginal candidiasis fabricated by numerous polymers which helps to enhance the adhesiveness or increase the residence time of fibers on the vaginal mucosa. Hence it increases the absorption and bioavailability of drugs, and decreases the frequency of drug administration. It was concluded that nanofibers outperformed with better mucoadhesive properties, sustained action, and better antifungal activity against C. albicans, and therefore can preferred more in VVC treatment in comparison to other conventional formulations. In the
future, if is scaled up at the clinical level it would be a remarkable breakthrough not only to reduce the health burden of VVC in women,’ but also for various biomedical applications.

ETHICAL STATEMENT

This study did not require any ethical approval, or informed consent for studies with human or animal subjects because this study only used published and pooled data.

FUNDING

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Author contribution

First and Second author- AV and AKS did primary and secondary screening and manuscript writing; Third and fourth author – TR and MA has draft the manuscript and table redrafting. Fifth and Sixth author – SP and KK has resolve the query and draft the methodology and manuscript.

Declaration on Interest

The author reports no declarations of interest.

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REFERENCES:


Table 01. Baseline characteristics of included studies of prepared electrospun nanofiber for Vulvovaginal candidiasis

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug loaded</th>
<th>Polymers used</th>
<th>Dimensions of nanofiber</th>
<th>Proposed application processes</th>
<th>Types of involved studies</th>
<th>Conclusion/Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Amphotericin – B</td>
<td>Poly(lactic-co-glycolic acid)</td>
<td>Unloaded fibers 582.3 ± 71.28 nm Loaded fibers 637.8 ± 93.81 nm</td>
<td>Flow rate: 3.6 ml/hr Voltage: 25 kV Collector distance: 10 cm Temperature: 27°C Humidity: 65%</td>
<td>Preclinical study</td>
<td>• Controlled release of amphotericin was observed • Vaginal fungal burden started to eradicate after 6 hours without any systemic toxicity.</td>
<td>Souza et al., 2018</td>
</tr>
<tr>
<td>2.</td>
<td>Fluconazole</td>
<td>PVA (8-12%)</td>
<td>200-350nm</td>
<td>Flow rate: 0.1-0.2 ml/hr Voltage: 8 to 12 kV Collector distance: 15 cm</td>
<td>Basic Research</td>
<td>• Localized delivery of fluconazole to treat VVC that showed 6 hours of sustained action.</td>
<td>Sharma et al., 2016</td>
</tr>
<tr>
<td>3.</td>
<td>Eugenol</td>
<td>Polyacrylonitri le (PAN) (15 weight %)</td>
<td>Unloaded fibers 127 ± 21 nm Loaded fibers 179 ± 70nm</td>
<td>Flow rate: 0.35 ml/hr Voltage: 18 kV Collector distance: 18 cm Temperature: 25°C Humidity: 35%</td>
<td>Basic Research</td>
<td>• Slow-release up to 150 hours • Suitable antifungal activity of eugenol was observed</td>
<td>Semnani et al., 2018</td>
</tr>
<tr>
<td>4.</td>
<td>Clotrimazole</td>
<td>PVA, Sodium alginate, Dextran</td>
<td>100-150 nm and 700-900 nm (Thicker fibers)</td>
<td>Flow rate: 0.7 ml/hr Voltage: 15-17 kV Collector distance: 12 cm</td>
<td>Basic Research</td>
<td>• Fiber and film of clotrimazole were prepared. • Comparing the two, fiber showed higher antifungal activity than film.</td>
<td>Najmeh et al., 2020</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>Polymer</td>
<td>Diameter</td>
<td>Flow rate</td>
<td>Voltage</td>
<td>Collector distance</td>
<td>Temp.</td>
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<tr>
<td>5</td>
<td>Benzydamine</td>
<td>Polyvinylpyrrolidone (PVP)</td>
<td>436 ± 155 nm to 557 ± 221 nm</td>
<td>(Not mentioned)</td>
<td></td>
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<tr>
<td>6</td>
<td>Eucalyptol</td>
<td>Gellan and Polyvinyl alcohol</td>
<td>NA</td>
<td>Flow rate: 0.1 ml/hr</td>
<td>Voltage: 18 kV</td>
<td>Collector distance: 18 cm</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Fluconazole and Ketoconazole</td>
<td>Polycaprolactone (PCL)/Polyvinylpyrrolidone (PVP)</td>
<td>656 nm</td>
<td>Flow rate: 0.2 ml/hr</td>
<td>Voltage: 12 kV</td>
<td>Collector distance: 18 cm</td>
<td>Temp.: 25°C</td>
</tr>
<tr>
<td>8</td>
<td>Sertaconazole Nitrate (SCZ)</td>
<td>Polyurethane/polyvinylpyrrolidone/silk</td>
<td>Diameters of SF/PU/PVP and SCZ/SF/PU/PVP nanofibers were 309.31 ± 92.49 nm and 257.00 ± 80.59 nm respectively</td>
<td>Flow rate: 1 ml/hr</td>
<td>Voltage: 15 kV</td>
<td>Collector distance: 15 cm</td>
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<td></td>
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<td>Infection in comparison to nanofibers without silk.</td>
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**Legends** - PVA- Polyvinyl alcohol; nm- Nanometer; ml- milliliter; hr- hour; kV- kilo Volt; NA- Not applicable; SF- Silk fibroin; PU- Polyurethane
Figure 01. PRISMA flow diagram showing relevant articles selection process
Figure.02. Electrospinning technique