# Biopolymer based nanofiber topical patches for the management of diabetic fungal wounds


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**DOCTOR OF PHILOSOPHY**

**By**

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# Summary & conclusions

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## 7.1 Summary

The idea behind this thesis was driven by the challenges associated with delayed wound healing in cases of infected diabetic fungal wounds. Diabetic wounds are prone to fungal infections, and fungi form biofilms over the affected sites, creating a microenvironment that further delays wound healing. The conventional treatments for diabetic wounds require repeated application of antifungal agents to the wound area. However, this repeated application is neither economical nor does it improve patient comfort. Therefore, the use of antifungal nanofiber mats could offer a better solution, due to capability of nanofibers to provide prolonged drug release, maintain gaseous exchange, absorb wound secretions, and promote wound healing.

For this two type types of nanofiber treatment were prepared and tested. First, includes the preparation of AgNP (silver nanoparticles) and LZNP (luliconazole loaded PLGA nanoparticles) loaded PVA/chitosan composite nanofiber (PVA/CH-AgNP-LZNP NF) and second one include luliconazole and naringenin loaded gelatin coated polycaprolactone nanofiber (GL-PCL-LTZ/NAR).

For the preparation of PVA/CH-AgNP-LZNP NF, a polymer blend of polyvinyl alcohol and chitosan were used. The polyvinyl alcohol as synthetic polymer carry wide application in tissue engineering due to its hydrophilic characteristics. But, PVA alone nanofiber could lacks necessary physical strength. So, polyvinyl alcohol composite nanofiber with chitosan will able to provide the necessary physical strength and help in bioadhesion. For PVA/CH-AgNP-LZNP NF preparation, initially AgNP and LZNP nanoparticles were prepared by green synthesis and solvent evaporation method, respectively. After that, prepared nanoparticle were loaded into the composite matrix of polyvinyl alcohol and chitosan nanofiber by electrospinning technique.

In second approach, GL-PCL-LTZ/NAR nanofiber was prepared by using the coaxial electrospinning. Inherently, the polycaprolactone due to hydrophobic nature allow direct loading of hydrophobic drugs within its matrix, but also limit its use in wound healing application. For this, polycaprolactone nanofibers were coated with gelatin layer (a hydrophilic nature polymer) to improve the hydrophilicity of the matrix.

After preparation all mats were tested using FTIR, XRD and DSC for solid state characterization; SEM, TEM and AFM for morphological characterization; contact angle, surface pH, water vapour capacity and water vapour transmission rate for physicochemical evaluation; antifungal, antibiofilm, drug release kinetics, and cytocompatibility study for i*n-vitro* characterization; and finally wound closure study and laser doppler study for *in-vivo* characterization.

The morphological evaluations showed interconnected smooth nanofiber morphology. The TEM analysis of PVA/CH-AgNP-LZNP NF showed successful loading of prepared nanoparticles within the matrix of PVA/CH nanofiber with AgNP in the core and LZNP as round bulbs over nanofiber surface. Similarly GL-PCL-LTZ/NAR nanofiber TEM analysis showed successful coating of gelatin over the surface of nanofiber and size in the nanorange.

The solid state study showed good physicochemical compatibility between drug and polymer in nanofiber matrix and successful entrapment of drug in nanofibers. The physicochemical characterization showed optimum surface pH (5.5-7 for topical application), good water absorption capacity and excellent vapour permeation capacity. These findings ensure effectiveness of prepared nanofiber preparations in absorbing wound secretions and maintaining gaseous exchange. Further, *in-vitro* characterization studies of nanofibers concluded that nanofibers have capacity to provide the prolonged release of

drugs (luliconazole and naringenin) for >10 days. Additionally, nanofibers were effective in eradicating *C.albican* and *C.tropicalis* fungal growth and maintaining Human epidermal keratinocytes cells proliferation. Finally, wound healing study nanofiber treatment groups demonstrated the superior wound healing response as compared to the untreated and marketed treatment (Luligel™ cream) in *Candida* infected diabetic wounds. This could be due to prolonged drugs release and antioxidant activity of nanofiber.

## 7.2 Conclusions

The present work involved the preparation and characterization of nanofiber matrices loaded with luliconazole and antioxidants for accelerated wound healing in infected diabetic wounds. AgNP and LZNP nanoparticle-loaded PVA/Chitosan composite nanofiber (PVA/CH-AgNP NF) and luliconazole and naringenin-loaded gelatin-coated polycaprolactone nanofiber (GL-PCL-LTZ/NAR) were prepared.

- The solid-state characterization of GL-PCL-LTZ/NAR and PVA/CH-AgNP-LZNP nanofibers revealed good physiochemical compatibility between the drug and polymer, as well as the entrapment of drugs within the nanofiber matrix.
- Morphological evaluation of the nanofibers concluded a smooth interconnected nanofiber morphology with diameters in the nanometer range.
- Surface pH, contact angle, water vapour transmission, and water uptake capacity studies of GL-PCL-LTZ/NAR and PVA/CH-AgNP-LZNP nanofibers showed that both nanofibers exhibited good surface hydrophilicity, enabling them to absorb wound secretions, maintain gaseous exchange, and promote healing.
- *In-vitro* drug release studies demonstrated that the nanofibers provided prolonged release of the drugs for over 10 days.

- *In-vitro* HaCaT cell cytocompatibility and haemolysis studies revealed that all nanofibers exhibited good cellular compatibility and supported cellular proliferation.
- *In-vivo* wound healing studies demonstrated that nanofiber formulations provided accelerated wound healing compared to gauze-treated or Luligel™-treated wounds.
- Histological examination of wound healing studies showed that nanofiber-treated groups exhibited better neovascularization and reepithelialisation, supporting the findings laser doppler and wound closure study.
- Laser Doppler studies concluded improved blood flow around the wound area in nanofiber treatment groups. One possible reason for this could be prolonged release of naringenin and AgNP, along with luliconazole, which ultimately resulted a better healing as observed in wound closure study.

Overall, this research underscores the superior efficacy of nanofiber-based wound treatments in promoting accelerated wound healing and reducing infection rates. These findings contribute valuable insights to the field of wound care, paving the way for the development of advanced therapeutic strategies aimed at enhancing patient outcomes and quality of life. Although initial results are promising, further investigations are needed to explore the long-term effects of nanofiber applications.