International Journal of
Nanotechnology and Allied Sciences

Research Article 2022 | Volume 6 | Issue 1 | 14-22

Open Access
Article Information

Received: June 23, 2021

Accepted: March 20, 2022

Online first: March 28, 2022

Published: May 31, 2022

Keywords
Electrospinning,
Poly Vinyl Alcohol,
Levofloxacin,
Bacterial susceptibility,
Sustained drug release.

Authors' Contribution
GMK conceived and designed the experiment, GMK, AC and MK carried out the experiments; GMK analyzed data and wrote the article; MAB gave final approval.

How to cite
Khuhro, G.M., Bhutto, M.A., Chachar, A., Kumar, M., 2022. Fabrication and Characterization of Antibacterial Biodegradable Polymeric Nanofibers of Polyvinyl Alcohol loaded with Levofloxacin for External Skin Infection. Int. J. Nanotechnol. Allied Sci., 6(1): 14-22.

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Fabrication and Characterization of Antibacterial Biodegradable Polymeric Nanofibers of Polyvinyl Alcohol loaded with Levofloxacin for External Skin Infection

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Abstract:
Electrospun nanofibrous membranes have gained great focus in medical research due to its biocompatibility and biodegradability. Research proposed that it is most advanced method of delivering drug to patient's body at a particular site of infection. Through this targeted delivery, there is increased rate of delivered drug to an infected body part; it may be organs, tissues or cells. As result, the action of recovery of infection is enhanced without causing any side effects. During present study sustainable electrospun nanofibers were fabricated via biodegradable synthetic polymer polyvinyl alcohol (PVA) loaded with antibiotic (Levofloxacin). Control (PVA) and antibiotic loaded (PVA/LVF) nanofibers were synthesized through simple electrospinning. Synthesized materials were characterized through scanned electron microscopy (SEM) for morphology that showed average fiber diameter size of 146.24±44.024 µm and 184.79±41.94 µm respectively. While for chemical characterization FTIR was carried out. Further antibacterial susceptibility was checked against *Escherichia coli* for different time period of incubation and maximum zone of inhibition was observed at 72 hours that was 29±0.25 mm as compared to 24 h and 48 h. While release rate of drug in artificial medium phosphate buffer saline (PBS) was measured by spectrophotometric method up to 72 hours. In the medium at initial stage nano fibers showed burst release of drug up to 24 hour, later on sustain release behavior was observed up to 72 h. From the findings it was suggested that synthesized material especially used in the synthesis of gauze for external skin infections, it could also be used in the synthesis of sutures, wound patches bandages and other biomedical applications.

INTRODUCTION

Nanotechnology has been widely investigated in drug delivery due to its convenience and high patient compliance (Pouton and Porter, 2008). Modern therapeutics is taking great advantages from controlled drug delivery system, in which polymers play important role. Normally, “control” means two different aspects. The first aspect is the capability to deliver a specific drug to a desired location, which could be a tissue, an organ, or an affected site within body. Another meaning of controlled drug delivery is managing the drug concentration within the system of circulation to avoid toxic side effects by maintaining drugs action.

Polymeric nanofibers are outstanding candidates for controlled drug delivery system. A wound is the result of breakage or defect in skin due to which normal anatomical structure and physiological function of skin tissue are disturbed (Boateng *et al.*, 2008). Infection is one of the factor which disturb the wound healing (Leeds *et al.*, 2017). The ideal wound dressing should have good biocompatibility, maintain the suitable microenvironment of tissue regeneration, protect the wound from the invasion of external pathogenic microorganisms and expedite tissue regeneration (Babu, 2000). This can be achieved by fusion of biomaterial with engineering technology (tissue engineering). This technology practically revolutionized human health. Sustained drug delivery which is achieved through drug incorporation in polymeric nanofibers (Sudhakar *et al.*, 2015). Several methods are used for this purpose including Self-assembly, Phase separation and electrospinning (Ketabchi *et al.*, 2017; Pham *et al.*, 2006) among which electrospinning has got magnificent importance in usage due to its available variability to be performed and easy handling while processing (Schiffman and Schauer, 2008; Xie *et al.*, 2008).

Electrospinning is a process by which fabrication of nanofibers from biocompatible and biodegradable polymers is carried out (Shin, 2007). Electrospinning system based on: spinneret, voltage supplier and collector as shown in figure1 (Pham *et al.*, 2006; Sill and Von Recum, 2008). Different factors affect quality, geometry and alignment of fibers like viscosity of solution, flow rate, distance between needle and collector, amount of voltage supplied, surface tension, temperature and humidity (Ma *et al.*, 2005; Yang *et al.*, 2005).

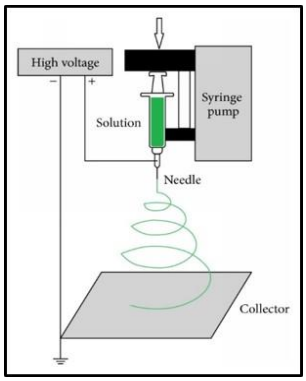

Fig. 1. Simple electrospinning

Different scientists have worked on targeted delivery system. They have proposed that it is most advanced method of delivering drug to patient’s body at a particular site of infection. Through this targeted delivery there is to increased rate of delivered drug to an infected body part it may be organs, tissues or cells. In the result, the action of recovery of infection is enhanced without causing any side effects. Basically, site drug delivery enhances the drug molecules availability directly at desired site within body. The main advantage of targeted drug delivery is the administration of drug at required site with its reduced dose and with fewer side effects. Targeted delivery system is under high consideration of research and development in the field of clinical and pharmaceutical as a back bone of therapeutics as well as diagnostic. Various types of drug carriers can be used in this advanced delivery system are soluble polymers, biodegradable microsphere polymers (synthetic and natural), neutrophils, fibroblasts, artificial cells, lipoproteins, liposomes, micelles and immune micelle (Rani and Paliwal, 2014).

Levofloxacin (LVF) is one of the synthetic water soluble antibacterial agent that belongs to fluoroquinolone class of antibiotics (Nelson *et*

al., 2007) It is used to treat severe or life threatening bacterial infections, where the other antibiotics fails to perform their action (Liu and Mulholland, 2005; MacDougall *et al.*, 2005). Levofloxacin inhibit the bacterial growth by inhibiting the function of enzymes like topoisomerase IV and DNA gyrase. The function of topoisomerase IV is to carry out the DNA separation that has been replicated (doubled) during bacterial division. So DNA could not separate properly in the result process of division is stopped.

Fundamental characteristics of polymer play an important role while working on a biomedical application, it should be harm free nature. Famous eco-friendly polymer, PVA-Poly vinyl alcohol is used (El-Shanshory *et al.*, 2015) which is used to synthesis of nanofibrous membrane. Poly Vinyl Alcohol is white or cream colored, synthetic and organic polymer which is hydrophilic in nature having neither odor nor taste. Now a day's targeted drug delivery has gained great interest of scientist in the medical field this is due to the some good properties of PVA, like biocompatible and biodegradable nature (Hill *et al.*, 1997). This study was carried out for the synthesis of antibacterial nanofibrous membrane and its effect against microorganisms. Synthesized material used in bandages or gauze to protect open wounds from attack of microorganisms and carry out fast healing process of wound.

MATERIALS AND METHODS

During research preparation of solution as well synthesis of nanofibers membranes through simple electrospinning process, antibacterial activity and release rate of drug was performed at Institute of Biotechnology and Genetic Engineering, University of Sindh Jamshoro, Pakistan. There is less hazarders even though it is approved from ethical committee of the institute.

Materials

All chemicals like PVA (Mw= 72,000 g/mol), and the degree of acetylation of PVA is 86.5 to 89%, drug Levofloxacin (Mw= 361.368 g/mol), standard antibiotic discs for bacterial susceptibility and phosphate-buffered saline (PBS) for sustained drug release were obtained from Dongnam Chemical Co. Ltd, Sigma-Aldrich (St. Loius, MO) and Merck etc. while all other chemicals were used high analytical grade throughout the study.

Preparation of PVA and PVA/LVF solutions

Polyvinyl alcohol water soluble polymer, prepared 8% PVA (control) solution in which no drug was added. While the other solution was prepared by the addition of antibiotic levofloxacin 5mg (0.05%) to polyvinyl alcohol solution (PVA/LVF), the viscosity of solutions was 467.3cP and 468.1cP respectively. During solution formation it was necessary to avoid any possible lump formation of PVA, which is highly insoluble. Solutions were placed on magnetic stirrer for 24 hours at room temperature.

Electrospinning

Nanofibrous membranes were prepared by using simple electrospinning technique. Polymeric solution loaded into a syringe, fix the syringe into the syringe pump flow rate was adjusted 1.0ml/hr. while other parameters were optimized as: Syringe was used 10 ml, stainless steel needle diameter 21 Gauge or 0.84 mm, Voltage supplied 17 KV, Distance b/w needle and collector was set 15 cm. As the solution started to emerge from the tip of the needle, drop of solution convert to form a Taylor cone due to presence of high electric field. Alternatively visualize the whipping motion and started the formation of fibers on the collector (aluminum foil). At the end fine nanofibrous membrane was formed on collector. During the process of fabrication the relative humidity was noted 40%. While another solution run in same way. Synthesized membranes were collected and stored them at optimal temperature.

Surface morphology of nanofibrous membranes

Morphological analysis of synthesized nanofibrous membranes were carried out by Scanned electron microscopy (JSM-638OLV)

(Bhutto *et al.*, 2016b). Prepared dried electrospun samples of PVA and PVA/LVF were coated through sputter coater with gold for 10s (twice) before scanning. Finally take out the image through SEM at accelerating voltage of 10 kV. Average nanofibers diameter was calculated by using software i-e Image J (specified for this purpose) by plotting histogram in unit of µm.

Chemical characterization of nanofibrous membranes

FTIR is an effective analytical instrument used for detecting the functional groups of samples as well as used to characterize the chemical group information. The chemical group conformation of control (PVA) and drug loaded (PVA/LVF) nanofibrous membranes were carried out by Fourier Transform Infrared Spectroscopy (ART-FTIR) (Nicolet 6700, Thermo Fisher USA) (Sun *et al.*, 2015). For each sample infrared measurements were calculated by an avatar 380 FTIR spectrometer at the transmission mode (32 scans) in the range of wave length 500-4000 cm^{-1}. Which were transformed in spectral line chart by using Origin software.

Antibacterial susceptibility of PVA/LVF nanofibrous membrane

Antibacterial activity of drug loaded sample (PVA/LVF) was carried by zone inhibition method (Kalwar *et al.*, 2017) against bacterial strain *Escherichia coli* using Luria-Bertani (LB) medium for their growth. *E.coli* culture was inoculated through streak plate method, than introduce freshly prepared autoclaved discs of PVA (control) and drug loaded (PVA/LVF) samples of size 1 cm in diameter in cultured petri plates under sterilized conditions using laminar air flow cabinet. Antibacterial activity of discs was checked at different time interval 24 h, 48 h and 72 h by comparing its size of inhibition zone with commercially available antibiotic discs zone.

Drug release profile of PVA/LVF electrospun nanofibrous membrane

Release behavior of antibiotic (Levofloxacin) from PVA/LVF sample was determined by using spectrophotometric method. Phosphate buffer saline (PBS) is used as in vitro release medium. Nanofibrous mat was taken in size of 2 x 2 cm and was placed in test tube having 10 ml PBS with pH 7.4 stored at 37°C. From this release medium after specific time interval 4 ml of solution was taken out and that same volume added in release medium with fresh PBS (Bhutto *et al.*, 2016a). Take out the absorbance at wave length of 290 nm. The released amount of LVF from nanofibrous mat at specific time interval was calculated by using standard graph of levofloxacin. Release profile of levofloxacin was finally presented in scattered line representation using following formula:

$$\text{Release (\%)} = \frac{Release\ of\ LVF}{Total\ loaded\ LVF} \times 100$$

RESULTS

Morphology of nanofibrous membranes

Synthesized nanofibrous membranes were scanned through electron microscope for their surface morphology. SEM images showed membranes were made of random nanofibers having no beaded structure with average nanofibrous diameter of 146.24±44.024 µm and 184.79±41.94 µm (Figure 2-A, B) for control (PVA) and drug loaded (PVA/LVF) respectively. The drug loaded nanofibers have larger average fiber diameter in comparison to control (PVA). It might be due to change in viscosity of solution by the addition of antibiotic (Levofloxacin). It was also reported that by maintaining same parameters for electrospinning under control environment, if the concentration of solution is changed it will affect the nanofibers diameter. Average thickness of nanofibers membranes was measured as 0.043mm and 0.048mm of PVA and PVA/LVF respectively.

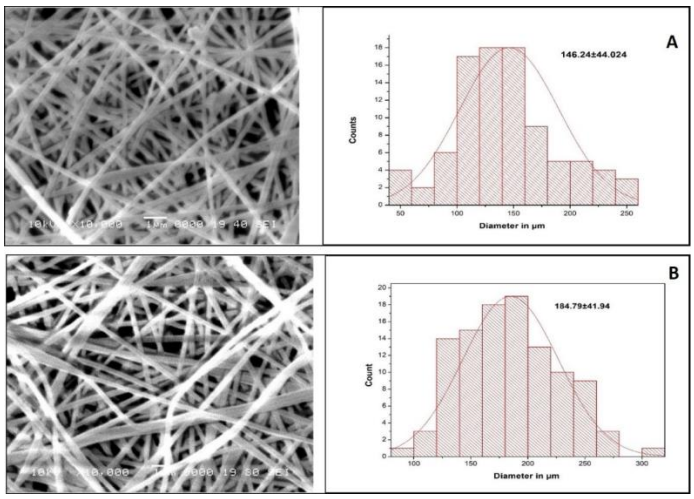

Fig. 2. (A) SEM image of control (PVA) nanofibrous membrane and its average fiber diameter. (B) SEM image of PVA/LVF nanofibrous membrane and its average fiber diameter.

Chemical Characterization nanofibrous membranes by FTIR

FTIR spectra of control (PVA) as well as antibiotic loaded sample (PVA/LVF) nanofibrous membranes were presented in Figure 3. Characteristic peaks of PVA spectrum is showing at 2910 cm^{-1} and 2941 cm^{-1} lies in the ranges of 2800 to 3200 cm^{-1}, which conforms the presence of OH group in PVA, which was also present in PVA/LVF samples. While pure antibiotic (Levofloxacin) was showing small stretching peaks at 1143 cm^{-1} and 1327 cm^{-1}, which were very much close to carboxylic acid group. Linkage of quinolone group with carboxylic acid caused a possible variance of peaks from pure carboxylic ranges. These peaks were shifted from 1143 cm^{-1} to 1141 cm^{-1} and 1327 cm^{-1} to 1328 cm^{-1} in antibiotic loaded sample. However, these shifting or stretching of peaks happened due to the blending of pure drug with polymer.

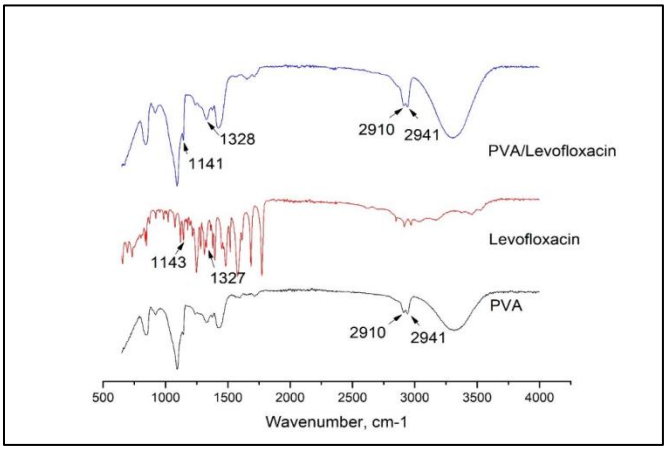

Fig. 3. FTIR spectrum of nanofibrous membranes of Control (PVA) and drug loaded (PVA/LVF) samples. While levofloxacin peaks in pure drug.

Antibacterial susceptibility

Antibacterial susceptibility possessed by nanofibrous membranes were examined against bacterial strains of *Escherichia coli*. Discs of nanofibrous mat (PVA/LVF) showed significant susceptibility against bacterial strains with formation of clear inhibition zones. Effect of levofloxacin depends on dosage. Here levofloxacin used is used in nanofibers only for external use for the treatment of skin infections. The larger zone was observed in PVA/LVF nanofibrous mat of 29 mm at 72 hours that was higher as compare to 24 h and 48 h as shown in figure 4. While it was observed that control (PVA) sample showed no antibacterial activity against *E. coli*, it was also cleared from literature that PVA has no antibacterial activity. Results confirmed that the PVA/LVF has higher activity, it is due to the presence of antibiotic inside the nanofibers. Additionally, it was cleared that sample possess excellent resistance up to 72 h as compare to initial durations like 24 and 48 h. It was due to continuous release behavior of drug from nanofibers for longer duration. Standard disc of levofloxacin was used for comparison, there was a significant resemblance in the result of standard disc as well as drug loaded Nanofibrous mat (PVA/LVF) but the major benefit was that drug loaded in polymer released for a longer time and showed antibacterial activity for a longer time. Results conclude that prepared antibiotic loaded nanofibrous mat could be recommended as potential material to be used in biomedical applications.

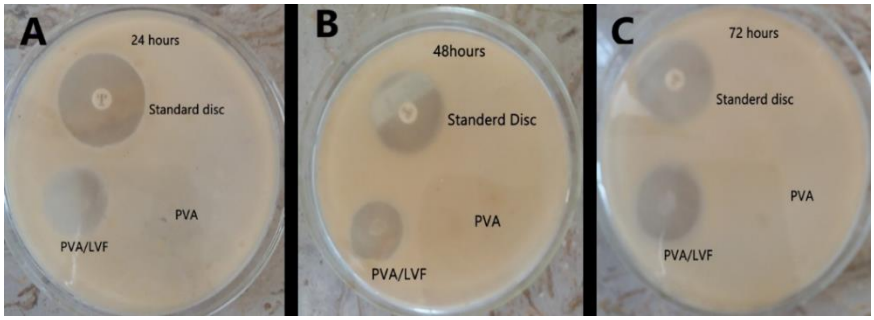

Fig. 4. Antibacterial activity of standard disc of levofloxacin and synthesized nanofibrous membranes of drug loaded (PVA/LVF) as well as control (PVA) nanofibrous mat at different time interval A. 24hr, B. 48hr and C. 72hr.

Levofloxacin release rate from PVA/LVF nanofibrous membrane

The release behavior of antibiotic (Levofloxacin) from nanofibers was totally depending on the nature of polymer, it may be hydrophobic or hydrophilic in nature. During this study hydrophilic polymer polyvinyl alcohol (PVA) was used. As for in vitro release behavior of PVA/LVF nanofibrous membrane were tested in PBS solution. During this study it was observed that PVA/LVF nanofibrous membrane showed burst release during initial 24 h, while after 24 hours sustain release was observed up to 72 h and on ward as in Figure 5. In the light of results of sustained drug release behavior of synthesized material is most applicable in biomedical application.

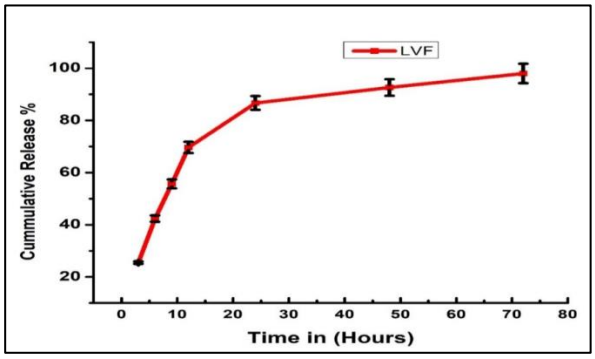

Fig. 5. Release rate of levofloxacin from nanofibrous membrane of PVA/LVF.

DISCUSSION

Nanofibrous membranes were prepared by using simple electrospinning technique. Synthesized nanofibrous membranes were scanned through electron microscope for their surface morphology. It was reported that by maintaining same parameters for electrospinning under control environment, if the concentration of solution is changed it will affect the nanofibers diameter (Zhao *et al.*, 2005), such type of findings were obtain during the study when drug was incorporated into the fibers, the fiber size was changed as compare to without drug loaded nanofibers. Drug conformation into fibers were checked by FTIR, where the shifting of peaks happened, these shifting or stretching of peaks happened due to the blending of pure drug with polymer, as like linkage of quinolone group with carboxylic acid causing a possible variance of peaks as reported earlier that shifting is possible due to shifting of functional groups (Trivedi and Vasudevan, 2007). Drug effect depends on the dosage of levofloxacin loaded into fibers, but the major benefit was that drug loaded in polymer released for a longer time and showed antibacterial activity for a longer time (Schaeffer, 2003). It was evidenced from the literature that release rate of drug from hydrophilic nanofibers is faster as compare to hydrophobic polymer based nanofibrous (Pelipenko *et al.*, 2015; Vrbata *et al.*, 2013). Release rate was calculated in artificial medium (PBS) which showed sustain release behavior. From the findings as well as suggested literature comparison the synthesized material most applicable in biomedical application (Jalvandi *et al.*, 2017). Synthesized material is able to use only for external treatment of skin infections.

CONCLUSION

During this research it was attempted to fabricate polymeric nanofibers loaded with antibiotic (Levofloxacin) that exhibits the antibacterial properties. Nanofibers membranes were fabricated through simple electrospinning process. During the study synthetic polymer polyvinyl alcohol (PVA) and the antibiotic was used to create nanofibrous mat. This was successfully characterized by SEM and FTIR, while for biological characterization antibacterial activity against *E.coli* was performed. Sustain release rate of antibiotic from nanofibers mat was also illustrated. These nanofibrous material especially used in the synthesis of gauze for external infections, it could also be used in the synthesis of sutures, wound patches bandages and other biomedical applications.

ACKNOWLEDGMENT

Many Thanks to Institute of Biotechnology and Genetic Engineering, University Of Sindh Jamshoro, which provide working space, material for nanofibers synthesis as well as instruments during this research work. In addition Thanks to Department of Metallurgy and Materials Engineering, Mehran University of Engineering and Technology Jamshoro, Pakistan, for Scanning Electron Microscopy (SEM).

CONFLICT OF INTEREST

The authors declare that this article's content has no conflict of interest.

Funding: None.

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