Bioactive Silvadur loaded polyacrylonitrile nanofibrous membranes for wound dressing applications

Maira Ayaz 1, Abdul Salam 1, Saif Ullah Khan 1, Muhammad Qamar Khan 1,∗, Tanveer Hussain 1 and Ick Soo Kim 1,∗

1 Nanotechnology Research Group, Department of Textile and Clothing, Faculty of Engineering and Technology, National Textile University Karachi Campus, Industrial Area Korangi, Karachi 74990, Pakistan
2 Department of Textile Engineering, Balochistan University of Information Technology Engineering and Management Sciences Quetta, 87650, Pakistan
3 Division of Frontier Fiber, Institute of Fiber Engineering, Interdisciplinary Cluster for Cutting Edge Research (ICCER), Faculty of Textile Sciences, Shinshu University, Tokida 3 15 1, Ueda, Nagano 386 8567, Japan

∗ Authors to whom any correspondence should be addressed.
E-mail: drqamar@ntu.edu.pk

Keywords: nanofibers, wound dressings, silvadur, nanomembranes, polymer

Abstract

Persistent wounds are the most problematic for the patient as well as for the health system. Skin wounds are most exposed to bacterial attacks, which not only cause wound infections but also slow down the healing process. There is a dire need to develop a better wound dressing or scaffold material that can increase the wound healing process. This study involves the development of electrospun nanofibers based on Silvadur-loaded polyacrylonitrile membranes. Samples were developed by using five different concentrations (2 wt%, 4 wt%, 6 wt%, 8 wt%, and 10 wt%) of Silvadur loaded in PAN solution. Resultant nanofibers were characterized by SEM, FTIR, XRD, and antibacterial tests. SEM analysis confirms that all the prepared electrospun nanofibrous membranes have smooth and bead-free surfaces. The average diameter of developed nanofibers lies in the range of 150 nm to 190 nm. It was confirmed that as the concentration of Silvadur increased the diameter of nanofibers also increased due to the increase in the viscosity of the electrospinning solution. FTIR interpretation confirms that the interaction between the PAN and Silvadur is physical, not chemical. XRD analysis reflects the crystallographic and macromolecular structure of prepared electrospun nanofibers. A qualitative antibacterial test was performed to check the antibacterial properties of prepared electrospun nanofibers against gram-negative bacteria (Escherichia Coli) and gram-positive bacteria (Staphylococcus Aureus). The result reveals that nanofibers loaded with the maximum concentration of Silvadur show the maximum antibacterial activity of 92.25% against Escherichia Coli and 98.52% against Staphylococcus Aureus. The higher antibacterial activity against gram-negative bacteria is due to the thinner cell wall as compared to the gram-positive bacteria.

1. Introduction

A wound is an injury to the living cells caused by various reasons like burning, accident, surgery, etc. Due to the presence of harmful bacteria in our atmosphere, every day millions of people suffer from wound infections that remain there for a long time [1]. In this scenario, there is a dire need to develop a better wound dressing or scaffold material that can increase the wound healing process. An ideal wound dressing should have appropriate mechanical properties, nontoxic, and biocompatible in nature with appropriate porosity for the exchange of nutrients and other gases [2, 3]. Polymeric-based nanofibrous membranes could be the best candidate for the development of the ideal wound dressing because they contain most of the properties that are required for an ideal wound dressing [4]. Various techniques have been used for the development of nanofibrous membranes such as drawing technique, template synthesis, phase separation technique, and self-assembly technique [5].
Every technique has its pros and cons, but these techniques cannot be used for bulk production. Out of these techniques, however, electrospinning is a fast production technique that varies from a single-fluid blending [6], coaxial [7], tri-axial, side-by-side [8], tri-layered side-by-side [9], and other complex processes. These processes are meaningful in creating complex nanostructures. However, the most popular process is still the single-fluid blending process, by which the functional ingredients can be encapsulated in the nanofibers through the co-blending solution. This process also holds great promise for production on a large scale [10]. The electrospinning technique usually involves a high voltage power supply, a collector for the collection of the nanofiber, and a spinneret for the extrusion of polymeric solution. Nanofibers of very fine diameters can be produced through this technique [11]. Polymeric electrospun nanofibrous membranes are widely used for biomedical and antibacterial applications [12]. Furthermore, polymeric nanofibrous membranes themselves do not possess antibacterial properties but can be imparted by using different antibacterial agents [13]. There are two most popular methods for imparting the antibacterial properties in the polymeric electrospun nanofibrous membranes. The first is the incorporation of the antibacterial agent within the polymeric solution before electrospinning [14] and the second one is the surface functionalization of polymeric electrospun nanofibrous membranes with antibacterial agents after the electrospinning process [15, 16]. Lots of researchers used various nanoparticles with different polymers combinations to impart the antibacterial properties in the nanofibrous membranes. Shalaby et al. developed polyacrylonitrile (PAN) based antibacterial nanofibers by using three different nanoparticles (CuO, Ag, and TiO2) and used these nanofibers for the inactivation of bacteria from the drinking water. They have claimed that by increasing the concentration of nanoparticles, the antibacterial activity of the nanofibers can be enhanced [17]. Li et al. prepared nanofibrous based wound dressings composed of silver nanoparticles/Dimethylglyoxyl Glycine/Cellulose acetate with sustained drug release ability and antibacterial properties. They have found that drug process is very slow up to 84 h, which is required for the wound dressings. Moreover these nanofibrous membranes showed obvious antibacterial activity against Bacillus subtilis (B. subtilis) and Escherichia coli (E. coli bacteria) [18]. Wang et al. prepared the silver-based nanofibers by dispersing the nanoparticles in the PAN solution before electrospinning process. They have found that Raman spectrum of the PAN nanofibers were changed after the dispersion of the Ag nanoparticles in the PAN solution and prepared electrospun nanofibers showed sufficient antibacterial activity [19]. Shalaby et al. synthesized nanoparticles (ZnO, CuO or Ag) decorated PAN nanofibers for the treatment of drinking water. The average diameter of prepared nanofibers lies in the range of 170 nm-250 nm and these fibers shows an excellent antibacterial activity against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) bacteria [17]. Ullah et al. conducted a comparative study on the PAN nanofibers functionalized with silver sulfadiazine through in situ and immersion process. For the in situ process silver sulfadiazine was directly entrapped in the electrospinning solution while for the immersion method nanofibers were functionalized by using sulfadiazine sodium salt and AgNO3. This study reveals that the nanofibers mat functionalized through immersion process possess excellent bacterial and structural properties [20].

Although lots of researchers used different nanoparticles to impart the antibacterial properties, either through in situ or surface functionalization methods. The problem with the use of the nanoparticles is that they peeled off over time due to which nanofibers lose their antibacterial and other functional properties. So, there is a dire need to provide some robust method for the development of durable antibacterial polymeric nanofibrous membranes. Herein, this study will present a robust way to develop efficient antibacterial polymeric nanofibrous membranes by using Silvadur Technology [21]. Silvadur is a patented material composed of a homogeneous mixture of silver ions and proprietary polymeric binder (acrylic polymer) which are completely soluble in water/ethanol solution [22]. In this material silver nitrate is the precursor source for silver ions. Further details for the Silvadur can be found in European Patent [23]. Developed nanofibers will have potential applications in wound dressings, surgical face masks, and protective clothing.

2. Materials and methods

2.1. Materials

The Polycrylonitrile (MW = 150 000 g mol−1) was used as a precursor material due to its high mechanical strength, high modulus, and high chemical stability. Polycrylonitrile was purchased from Sigma Aldrich. N, N-Dimethylformamide (DMF)(Reagent Grade, MW = 73.1 g mol−1) was used as a solvent and purchased from Sigma Aldrich. Silvadur 930 Flex (dispersion of silver ions) was used as an antibacterial agent and purchased from iTextiles Pvt. Ltd. All the materials involved in this study were used without any modification or purification.
2.2. Method

(preparation of electrospinning solution and electrospun nanofibers)

Five different solutions were prepared for the development of electrospun nanofibers at various concentrations of Silvadur. Pure PAN solution was prepared as follows: 1 g of PAN was mixed in the 9 g of DMF solvent and stirred for about 8 h to get a homogeneous solution by maintaining the stirring speed of 500 rpm on a hotplate magnetic stirrer. A similar process was repeated for the preparation of Silvadur/PAN nanocomposites fibers. However, the concentration of Silvadur varied in each solution (2 wt%, 4 wt%, 6 wt%, 8 wt%, 10 wt%) as shown in table 1, and stirred for about 10 h to obtain a homogeneous solution. To obtain the pure PAN, PAN/Silvadur electrospun nanofibers, the prepared solutions were loaded in a disposable syringe of 10 ml. Placed the copper wire inside the syringe, this wire was acting as a positive electrode. The voltage of 12 kV was applied through the high voltage supply resulting in the formation of electrospun nanofibers. The prepared electrospun nanofibers were collected on the aluminium foil, which was wrapped onto the rotating collector. The distance between the tip of the syringe and the collector was 15 cm and the flow rate was maintained at 1 mL/min. Figure 1, indicates the illustration scheme to produce electrospun nanofibers.

3. Characterization techniques

The prepared electrospun polymeric nanofibrous membranes were tested through different characterization techniques. Scanning Electron Microscopy (JSM-5300) with an operative voltage of 12 kV was used to analyze the surface morphology of the prepared nanofibers. The diameter of prepared electrospun nanofibers was calculated by using image J (image analysis software, version 1.49). To investigate the chemical interaction between the PAN nanofiber and Silvadur, Fourier Transform Infrared (FTIR) analysis was performed. The spectra were scanned between 400 cm\(^{-1}\) to 4000 cm\(^{-1}\). The crystallographic and macrostructure of the nanofibers were analyzed by x-ray diffraction (XRD) analysis. Qualitative antibacterial analysis was used to check the antibacterial activity of pure PAN nanofibers and Silvadur-loaded PAN nanofibers. AATCC-100 (the American Association of Textile Chemists and Colorists) test standard method was used for this purpose while the antibacterial activity of nanofibers is tested against Escherichia Coli (gram-negative bacteria) and Staphylococcus Aureus (gram-positive) bacteria. The detailed method of antibacterial study is described in our previous study [14].

| Sr No. | Percentage of SD (Wt%) | PAN (g) | SD (g) | DMF (g) |
|-------|------------------------|---------|--------|---------|
| 1     | 0%                     | 1       | 0      | 9       |
| 2     | 2%                     | 0.98    | 0.02   | 9       |
| 3     | 4%                     | 0.96    | 0.04   | 9       |
| 4     | 6%                     | 0.94    | 0.06   | 9       |
| 5     | 8%                     | 0.92    | 0.08   | 9       |
| 6     | 10%                    | 0.9     | 0.1    | 9       |
4. Results and discussion

4.1. Surface analysis and diameter of polymeric nanofibrous membranes

To investigate the surface morphology of polymeric nanofibrous membranes SEM analysis was performed. Figure 2 represents the surface morphology of prepared electrospun nanofibers. The surface of all the prepared nanofibers is smooth and well aligned without any formation of beads as shown in figure 2(A-F). On the other hand, the average diameter of prepared electrospun nanofibers was calculated with the help of image J software [14]. For each sample, thirty readings were taken from different areas and the average diameter was measured by using the histogram. The calculated average diameter for the pure PAN nano fiber was 152.94 nm with a standard deviation of 24.43 as shown in figure 2A. The maximum diameter of 187.91 nm with a standard deviation of 8.59 was observed in the case of nanofiber loaded with a maximum concentration (10 wt %) of Silvadur as shown in figure 2(F). Whereas, the average diameter for the nanofibers (B-E) was 167.12 nm, 176.34 nm, 183.67 nm, and 185.05 nm with the standard deviation of 12.02, 12.42, 19.62, and 8.59 respectively. It was observed that the average diameter of the prepared nanofibers was affected by the concentration of Silvadur, as the concentration of Silvadur increases the diameter of the nanofibers also get increased. This could be explained with the help of two possibilities. Firstly, as the concentration of the Silvadur increases, the viscosity of the electrospinning solution may increase due to which the resultant nanofibers may have coarser diameter as compared to pure PAN nanofibers [24–26]. The second reason could be explained by the help of ionic repulsion, as the Silvadur contains silver ions with similar charges. Due to these similar charges the repulsive forces may be generated between the silver ions within the polymeric solution leading to the formation of coarse nanofibers [27].

4.2. Chemical interaction (FTIR) of prepared electrospun nanofibers

The chemical interaction of pure PAN nanofibers and 10 wt % loaded PAN nanofibers were determined by using Silvadur. Figure 3 corresponds to the FTIR analysis of pure Silvadur which contains silver nitrate as a major source of the silver ions. The broad peak appears between 3000 cm⁻¹ to 3500 cm⁻¹ corresponding to –OH bending due to the presence of water content in the Silvadur. The peak appears at 1633 cm⁻¹ N–H band due to the presence of silver nitrate. In the spectrum, the peak appears at 2364 cm⁻¹ corresponding to the O=–C=–O band [28]. Figure 4 indicates the FTIR spectra of nanofibers i.e. pure PAN nanofibers and 10 wt % Silvadur PAN nanofibers. In both spectra (A and B) there is a broader band which appears in the range of 3300 cm⁻¹ to 3500 cm⁻¹ corresponding to the hydroxyl (–OH) groups. The intensity of this band is higher in the case of figure 3 than figure 4. This might be due to the presence of silver Ag⁺ ions, these ions might be responsible to attract more and more hydroxyl (–OH) groups from the atmospheric moisture resulting in the higher peak intensity [29]. Moreover, in figure 4 the characteristic peaks appear at 2918 cm⁻¹, 2850 cm⁻¹ corresponds to the C–H stretching. In both spectra, the characteristic peaks appear at 2244 cm⁻¹ representing the –C≡N stretching due to the presence of nitrile groups in the PAN. The peaks appearing at 1732 cm⁻¹, 1598 cm⁻¹, and 1454 cm⁻¹ were related to –C=O, –N–O stretching, and O–H bending respectively. It is clear from figure 4, that both spectra (A and B) are quite similar to each other and no addition peak appear due to the addition of Silvadur, this is because of the very low quantity of Silvadure in the nanofibers that might not be detectable by FTIR [30, 31]. We are pretty sure that all the interaction between Silvadur and PAN is physical interaction [32].
4.3. XRD analysis of prepared electrospun nanofibers

Crystallographic and macromolecular structures of prepared electrospun nanofibers were investigated by XRD analysis. In figures 5(A)–(F), all the spectra show strong and weak diffraction peaks at an angle of 28.3° and 16.9° respectively. Such peaks correspond to the crystallographic planes (110) and (100) of PAN nanofibers respectively [33]. In the case of spectra (B–F), the diffraction peaks appear at 31.53°, 34.37°, 36.45°, and 56.75° indicating the presence of silver ions in the prepared electrospun nanofibers. These peaks correspond to the silver’s crystallographic planes (210), (111), (200), and (142) respectively. Moreover, such peaks do not appear in the pure PAN spectrum. From XRD spectra, the sharpness of the peaks depends upon the concentration of the Silvadur. As the concentration of Silvadur increases, the sharpness of the peaks got also increase, which suggests that a large number of silver ions are entrapped within the polymeric solution as a result the crystallinity of the nanofibers change considerably.

4.4. Antibacterial activity

Pure PAN electrospun Nanofibers and the electrospun nanofibers loaded with different concentration of Silvadur were tested for antibacterial activity. Antibacterial activity of prepared electrospun nanofibers was evaluated against Gram-negative bacteria (Escherichia Coli) and gram-positive bacteria (Staphylococcus Aureus). Qualitative analysis was performed by following AATCC 100 test standard method. Figure 6(A) shows that pure PAN nanofibers did not show any antibacterial activity in both cases against gram-negative bacteria or gram-positive bacteria. However, the antibacterial activity of nanofibers increases as the concentration of Silvadur increases. The nanofibers loaded with 10 wt% Silvadur show the maximum antibacterial activity of 92.25% and 98.52% against gram-negative bacteria and gram-positive bacteria respectively. Nanofibers loaded
with Silvadur provide the reactive sites for bacterial interaction due to the presence of silver ions [34]. These silver ions make the nanofibers a bacteriostatic agent, due to the presence of the positive charge silver ions on the surface of the nanofibers. Such positively charged ions get accumulated on the surface of the negatively charged bacteria through a strong electrostatic force of attraction. As a result of this accumulation, the lipid bilayer gets ruptured causing the damage of cell wall of bacteria, ultimately yielding to death [35]. From the figure 6, it can be seen that Silvadur nanofibers show high antibacterial activity in the case of gram-negative bacteria as compared to gram-positive bacteria, this is because the cell membrane of gram-negative bacteria (Escherichia coli) is much thicker than the gram-positive bacteria (Staphylococcus aureus) [36, 37]. Figure 7, shows the antibacterial mechanism of prepared polymeric nanofibrous membranes.
5. Conclusions

This study is aimed to develop the Silvadur-loaded PAN electrospun nanofibrous membranes for wound dressing and personal protective (surgical gowns, face masks) applications by using the electrospinning technique. The developed polymeric electrospun nanofibrous membranes possess a smooth surface with an average diameter lies in the range of 150 nm to 190 nm. Furthermore, these fibers show excellent antibacterial activity up to 92.25% in the case of Gram-negative (Escherichia Coli) bacteria and 98.52% in the case of gram-positive (Staphylococcus Aureus) bacteria. Hence Silvadur has great potential to kill the bacteria due to the presence of silver ions. The developed Silvadur-based polymeric nanofibrous membranes have a great potential for wound dressings and personal protective (face masks, surgical gowns) applications.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Funding

This research received no external funding.

Conflicts of interest

The authors declare no conflicts of interest.

ORCID iDs

Muhammad Qamar Khan @ https://orcid.org/0000-0002-1680-1588
Ick Soo Kim @ https://orcid.org/0000-0003-2244-4369

References

[1] Shetty V et al 2020 Oral soft tissue wound healing Oral and Maxillofacial Surgery in Dogs and Cats (Amsterdam: Elsevier) 1–5.e1
[2] Loh Q L et al 2013 Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size Tissue Eng. Part B Rev. 19 485–502
[3] Memic A et al 2019 Latest progress in electrospun nanofibers for wound healing applications ACS Appl. Bio Mater. 2 952–69
[4] Guo Y et al 2021 Aramid nanofibers reinforced polyaniline alcohol/tannic acid hydrogel with improved mechanical and antibacterial properties for potential application as wound dressing J. Mech. Behav. Biomater. Mater. 118 104452
[5] RMP et al 2021 Nanofibers—a newer technology Res. J. Pharm. Technol. 14 2321–7
[6] Mehdi M et al 2021 Fabrication and characterization of irizatrazipan loaded pullulan nanofibers as oral fast–dissolving drug system Mater. Res. Express 8 055404
[7] Meraz-Dávila S et al Apr. 2021 Challenges and advantages of electrospun nanofibers in agriculture: a review Mater. Res. Express 8 042001
[8] Xu H et al 2022 Electrospun hierarchical structural films for effective wound healing Biomater. Adv. 136 212795
[9] Liu H et al 2022 Electrospun structural nanohybrids combining three composites for fast felicite delivery Adv. Compos. Hybrid Mater. 5 1017–29
[10] Salam A et al 2020 In-vitro assessment of appropriate hydrophilic scaffolds by co-electrospinning of poly(1,4 cyclohexane isosorbide terephthalate)/polyvinyl alcohol Sci. Rep. 10 19751
[11] Sišková A O et al 2021 Circulatory management of polymer waste: recycling into fine fibers and their applications Materials 14 4694
[12] Abdo G G et al 2021 A comprehensive review summarizing the recent biomedical applications of functionalized carbon nanofibers J. Biomed. Mater. Res. Part B Appl. Biomater. 109 1893–908
[13] Yu W et al 2021 Graphene oxide–silver nanocomposites embedded nanofiber core–spin yarns for durable antibacterial textiles J. Coll. Interface Sci. 584 164–73
[14] Salam A et al 2021 Electrospun nanofiber–based viroblock/ZnO/PAN hybrid antiviral nanocomposite for personal protective applications Nanomaterials 11 2208
[15] Borah R et al 2021 Surface–functionalized conducting nanofibers for electrically stimulated neural cell function Biomacromolecules 22 594–611
[16] Hamdan N et al 2021 Functionalized antimicrobial nanofibers: design criteria and recent advances J. Funct. Biomater. 12 59
[17] Shalaby T et al 2018 Electrospun nanofibrous hybrid composites membranes for highly efficient antibacterial activity Ecotoxicol. Environ. Saf. 162 354–64
[18] Li C et al 2022 Antibacterial properties and drug release study of cellulose acetate nanofibers containing ear-like Ag NPs and Dimethylsulphide/glycerin/beta-cyclodextrin Appl. Surf. Sci. 590 153132
[19] Wang Y et al 2005 Preparation of silver nanoparticles dispersed in polyacrylonitrile nanofiber film spun by electrospinning Mater. Lett. 59 3046–9
[20] Ullah S et al 2019 Antibacterial properties of in situ and surface functionalized impregnation of silver sulfadiazine in polyacrylonitrile nanofiber mats Int. J. Nanomedicine 14 2693–703
Silvadur® website https://pulcra-chemicals.com/silvadur (accessed May 30, 2022)
SILVADUR™ Antimicrobial Technology versus Nano Silvers — Wazoodle Fabrics [Online] Available: https://wazoodle.com/blogs/news/silvadur-antimicrobial-technology-versus-nano-silvers

Ghosh T et al. 2006 Antimicrobial fabric finish 1 1–13
Kharaghani D et al. 2018 Preparation and in-vitro assessment of hierarchal organized antibacterial breath mask based on polycrylonitrile/silver (PAN/AgNPs) nanofiber Nanomaterials 8 461
Fang S et al. 2015 Preparation of ZnO(AI, La)/polyacrylonitrile (PAN) nonwovens with low infrared emissivity via electrospinning Mater. Lett. 143 120–3
Yu H et al. 2013 Fabrication of Ag,PO₄—PAN composite nanofibers for photocatalytic applications CrystEngComm 15 4802
Shokraei S et al. 2021 Fabrication and characterization of chitosan/kefiran electrospun nanofibers for tissue engineering applications J. Appl. Polym. Sci. 138 50547
Huq M A. 2020 Green synthesis of silver nanoparticles using pseudoduganella eburnea MAHUQ-39 and their antimicrobial mechanisms investigation against drug resistant human pathogens Int. J. Mol. Sci. 21 1510
Shah A P et al. 2019 High performance visible light photocatalysis of electrospun PAN/ZnO hybrid nanofibers J. Ind. Eng. Chem. 77 154–65
Costescu A et al. 2013 Fabrication, characterization, and antimicrobial activity, evaluation of low silver concentrations in silver-doped hydroxyapatite nanoparticles J. Nanomater. 2013 194854
Liu X et al. 2013 Synthesis of silver-incorporated hydroxyapatite nanocomposites for antimicrobial implant coatings Appl. Surf. Sci. 273 748–57
Karim S A et al. 2019 Visible light photocatalytic activity of PAN-CNTs/ZnO-NH2 electrospun nanofibers J. Alloys Compd. 772 650–5
Pawar O et al. 2016 Green synthesis of silicon nanoparticles from purple acid phosphatase apoenzyme isolated from a new source Limonia acidissima J. Exp. Nanosci. 11 28–37
Gao Y et al. 2014 Electrospun antibacterial nanofibers: production, activity, and in vivo applications J. Appl. Polym. Sci. 131
Villarreal-Gómez L J et al. 2021 Antimicrobial effect of electrospun nanofibers loaded with silver nanoparticles: influence of Ag incorporation method J. Nanomater. 2021 1–15
Lawrence R et al. 2009 Isolation, purification and evaluation of antibacterial agents from Aloe Vera Brazilian J. Microbiol. 40 906–915
Khanzada H et al. 2020 Fabrication of Promising antimicrobial aloe vera/PVA electrospun nanofibers for protective clothing Materials (Basel) 13 3884