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Antibacterial Activity of Polyvinylidene Fluoride/polyethylene Oxide Nanofibers Loaded with *Azithromycin* for Wound Dressing

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Abstract: *Treating skin injuries remains challenging due to issues like wound infections. In this study, polyvinylidene fluoride (PVDF)/polyethylene oxide (PEO)/azithromycin (AZ) composite nanofibers were prepared using electrospinning to reduce bacterial infections in skin wounds. The surface morphology, chemical structure, and hydrophilicity of the nanofibers were characterized using scanning electron microscopy, Fourier transform infrared spectroscopy, and contact angle measurements, respectively. Antibacterial performance tests revealed that increasing the AZ dosage expanded the antibacterial zone, indicating improved effectiveness. Furthermore, experiments on rat skin infections showed that the PVDF/PEO/AZ membrane inhibited suppuration at S. aureus-infected wound sites. These findings demonstrate the potential of AZ-loaded PVDF/PEO nanofiber membranes as effective antibacterial dressing.*

Keywords*: electrospinning, PVDF, PEO, azithromycin, antibacterial, nanofibers*

1. Introduction

Skin injury occurs when the skin is damaged by external factors such as cuts, burns, and abrasions. Bacterial contamination significantly affects wound healing [1, 2]. Significant research efforts focus on developing and producing effective antibacterial wound dressings [3,4]. Modern wound dressings incorporate various materials like natural and synthetic polymers, herbal extracts [5], antibiotic [6], and metal ions and oxides [7]. These dressings come in forms like films, sponges, and hydrogels, designed to enhance wound healing and improve overall quality.

Electrospinning is a widely used, cost-effective polymer processing technique that forms nanofiber dressings by stretching polymer solutions or molten polymers into nano-sized fibers under an electric field [8]. Electrospinning produces dressings with diameters ranging from tens to hundreds of nanometers and provides multiple advantages for skin injury repair. The high specific surface area and porous structure mimic the extracellular matrix, promoting cell adhesion, growth, and differentiation [9-11]. These dressings also provide good permeability and breathability, preventing water vapor accumulation while promoting oxygen and nutrient transport. PVDF (polyvinylidene fluoride) and PEO (polyethylene glycol) are commonly used in electrospinning [12, 13]. PVDF exhibits good bio-compatibility and biological stability, does not easily cause immune reactions or tissue rejection *in vivo*, and provides strong chemical resistance and mechanical stability [14]. PEO offers good solubility and stretchability, enabling the formation of uniformly dispersed solutions with other polymers to create nanofibers with high tensile strength and plasticity [15-17]. Adding antibacterial agents to PVDF or PEO solutions produces dressings with antibacterial properties [18-20]. Incorporating PEO into PVDF solution improves the hydrophilicity of electrospun fibers, enhancing permeability, adsorption performance, and moisture regulation of electrospun dressings.

Azithromycin (AZ) is a broad-spectrum macrolide antibiotic with antibacterial properties, used to treat various infectious diseases by binding to ribosomes, preventing protein synthesis, and causing bacterial death [21-23]. AZ-loaded nanomaterials provided the advantage of sustained and controlled drug release, maintaining therapeutic blood concentrations for an extended period [24].

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Nanotechnology has been used to enhance the efficacy of antibiotics against specific microorganisms, improving activity levels [25]. PVDF/PEO/AZ-based polymer nano-dressings may provide an advanced wound care solution that combines the antimicrobial properties of AZ with the mechanical strength and biocompatibility of PVDF and PEO. This AZ-loaded nanocomposite dressing ensures sustained release at the wound site, accelerates the healing process, prevents infection, and reduces inflammation in various types of wounds. Furthermore, the versatility of these nanodressings allows them to be tailored for specific wound types and conditions, opening up new avenues for research and development in regenerative medicine and wound care.

In this study, PVDF/PEO/AZ composite nanofibers were prepared using electrospinning technology, and their antibacterial performance was evaluated (Figure 1). To assess the physical and biological properties of the nanofiber membranes, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and contact angle (CA) measurements of PVDF/PEO and PVDF/PEO/AZ membranes have been characterized. The antibacterial efficacy of these membranes against gram-positive and gram-negative bacterias were then investigated. Finally, their antibacterial activity was verified in vivo *S. aureus* infection model.

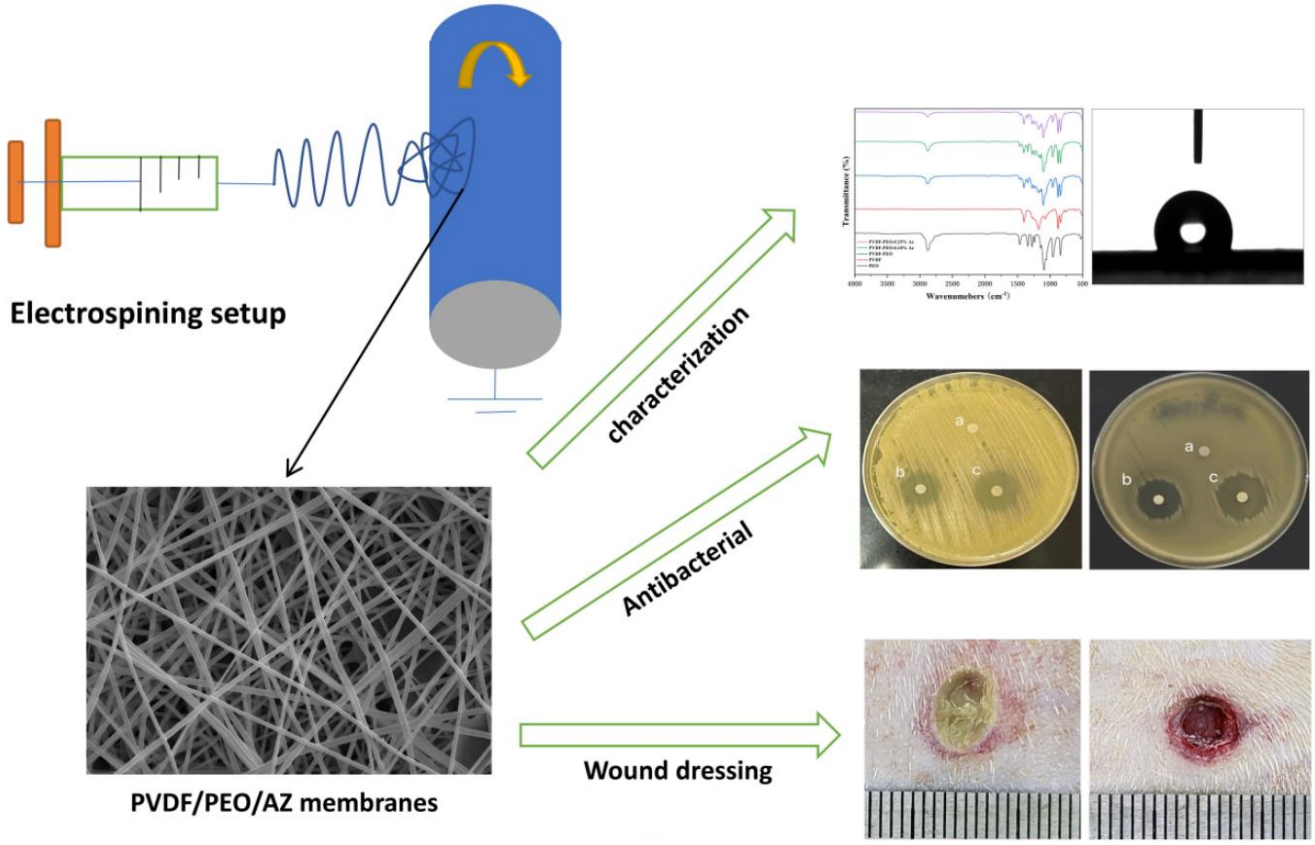

Figure 1. Schematic representation of electrospun PVDF/PEO/AZ membranes with antibacterial activity for wound dressing

2. Materials and methods

2.1. Materials

PVDF (molecular weight 1,000,000) was purchased from Changsha Nanoapparatus Co., Ltd. PEO (molecular weight 10,000) was purchased from Dongguang Zhan Yang Company. Azithromycin and N, N-dimethylformamide (DMF) were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. Yeast extract was obtained from OXOID UK. Tryptone, agar, and NaCl were obtained from Shanghai

Sangon Biotech Co., Ltd. *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), and *E. coli* (ATCC 25922) were all obtained from the Key Laboratory of Molecular Biology of Shaoyang University.

2.2. Preparation of the nanofiber membranes

The PVDF and PEO dissolved in DMF were prepared into a PVDF/PEO blend solution at ratios of 0.1:1, 0.5:1, 1:1, and 1.5:1 (m/m). AZ (0.02 g or 0.05 g) was added to the PVDF/PEO blend solution at a ratio of 1.5:1, respectively, to prepare PVDF/PEO/AZ spinning solutions with AZ concentrations of 0.1% and 0.25%, and then placed in a magnetic stirrer for 12 h before use.

The volume of 15 mL prepared spinning solution into an electrospinning machine, with a flow rate of 1 mL/h, a voltage of 10.03 kV, a round-trip distance of 50 mm, and a spinning time of 15 h. After electrospinning, the edges of the obtained nanofiber membranes were trimmed to leave a uniform thickness in the middle. The membranes were then placed on an ultraclean workbench for UV sterilization for 1 h, and subsequently stored in a plastic bag.

2.3. Characterization of the nanofiber membranes

The nanofiber membranes were coated using a Cressington 208HR high-resolution sputter coater at 20 mA for 120 s and then observed by SEM (Thermo Scientific Apreo 2C). The average diameter and distribution of the nanofibers were calculated using nano measure processing software, based on at least 50 randomly selected fibers from SEM micrographs.

The nanofiber membranes were first crushed and combined with potassium bromide powder to achieve uniformity. The mixture was further ground to ensure homogeneity, then compressed into pellets. The chemical structure of these pellets was analyzed using FTIR (Germany Bruker Optic GmbH).

Water contact angle measurements (OCA 20, Germany Dataphysics) were performed at room temperature using ultrapure water as the test liquid. A 3 μL droplet was carefully placed vertically onto the sample surface. Once in focus, an image was captured to measure and determine the contact angle accurately.

2.4. Antibacterial experiment

Cut the PVDF/PEO, PVDF/PEO/0.1%AZ, and PVDF/PEO/0.25%AZ membranes into 5 mm diameter circular discs and sterilize them with ultraviolet light for later use.Different fiber sheets were then lightly applied to the surface of agar plates coated with bacterial strains (*S. aureus, B. subtilis*, and *E. coli*), incubated at 37°C for 24 h, and then removed. The diameter of the antibacterial zone was measured after incubation.

2.5. Animal experiments

Select 220 g Sprague-Dawley (SD) rats to create an animal infection model of *S. aureus*. First, anesthetize the rats with pentobarbital sodium at a dose of 60-70 mg/kg and shave the hair on their backs. Then, use a skin puncher to create a 5 mm diameter circular wound extending into the subcutaneous tissue. Inject 20 μL of *S. aureus* suspension into each wound. The control group used medical sterile transparent patches, while the treatment group used 1 cm diameter PVDF/PEO fiber membranes, PVDF/PEO/0.1%AZ, and PVDF/PEO/0.25%AZ nanofiber membranes. After 2 days, remove the fibrous membrane and observe the formation of suppuration at the injury site. All animal experiments in this study were approved by the Ethics Committee of Shaoyang University.

3. Results and discussions

3.1. Morphology of the nanofiber membranes

To select the optimal PVDF/PEO ratio, the morphology of PVDF/PEO nanofibers was analyzed at ratios of 0.1:1, 0.5:1, 1:1, and 1.5:1 (Figure 2A). PVDF/PEO could not form a fibrous structure at ratios of 0.1:1 and 0.5:1, resulting in microspheres. At ratios of 1:1 and 1.5:1, PVDF/PEO formed intact and continuous fibers without bead structures, and the diameter of fibers increases with the concentration of

PVDF while the diameter was 0.29 ± 0.06 μM at 1:1 and the diameter was 0.40 ± 0.11 μM at 1.5:1 (Figure 2B). These findings indicated that the PVDF concentration in the solution was crucial for determining the nanofibers' morphology and size. Increasing PVDF content significantly improved the structural integrity of the fibers. Therefore, a PVDF/PEO ratio of 1.5:1 was selected for fiber spinning as the optimal carrier for AZ.

After adding AZ to PVDF/PEO, PVDF/PEO/0.1%AZ nanofibers maintained clear boundaries, while PVDF/PEO/0.25%AZ nanofibers stucked together, decreasing the uniformity of fiber diameter distribution (Figure 2C). Additionally, adding AZ made the fiber surface rougher and increased the fiber diameter. The diameter of PVDF/PEO/0.1%AZ was 0.52 ± 0.17 μM, while that of PVDF/PEO/0.25%AZ was 0.82 ± 0.23 μM (Figure 2D).

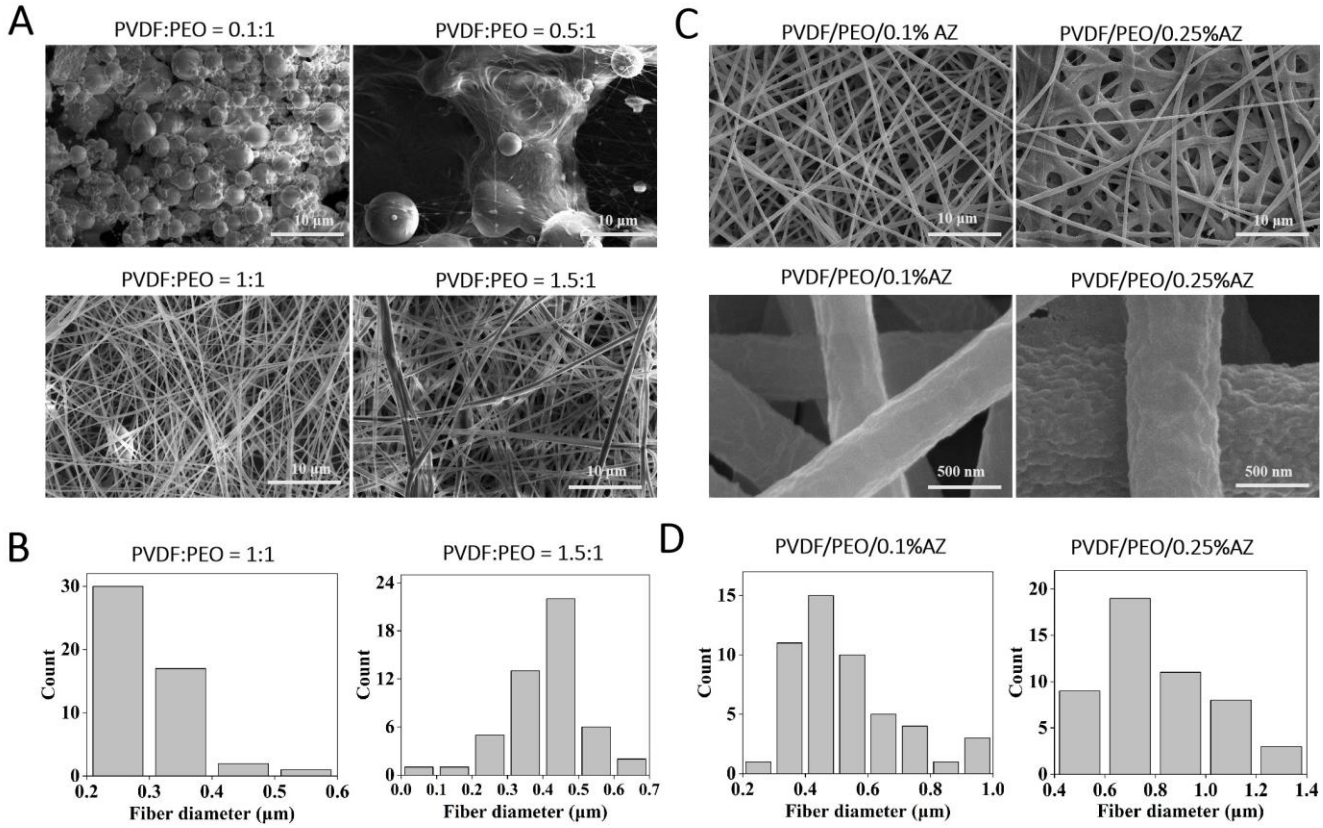

Figure 2. The SEM images and diameter distribution of different PVDF/PEO ratio, PVDF/PEO/0.1%AZ and PVDF/PEO/0.25%AZ. The SEM images of different PVDF/PEO ratio (A), PVDF/PEO/0.1%AZ and PVDF/PEO/0.25%AZ (C), Histograms of the nanofiber diameters at the PVDF/PEO ratio of 1:1 and 1.5:1 (B), PVDF/PEO/0.1%AZ and PVDF/PEO/0.25%AZ nanofiber (D)

3.2. Infrared of the nanofiber membranes

Figure 3 showed the infrared spectra of PEO, PVDF, PVDF/PEO, PVDF/PEO/0.1%AZ, and PVDF/PEO/0.25%AZ membranes. The absorption peak at 838 cm^{-1} corresponded to the bending vibrations of -CH bonds. The peak at 1400 cm^{-1} corresponded to the anti-symmetric stretching vibration of C-F bonds, while the peak at 1176 cm^{-1} corresponded to the symmetric stretching vibration of C-F bonds. These findings were consistent with the known infrared absorption characteristics of PVDF. The absorption peaks at 1092 cm^{-1} and 954 cm^{-1} were attributed to the anti-symmetric and symmetric stretching vibrations of C-O-C bonds, respectively. The peak at 2878 cm^{-1} corresponded to the stretching vibration of $-CH_2$ bonds. These findings aligned with the known infrared absorption characteristics of PEO. In the PVDF/PEO/0.1%AZ nanofiber membrane, the peaks near 1361 cm^{-1} were attributed to the C-N stretching vibration of AZ. After changing the AZ addition ratio, there was no significant change in

the position of each characteristic peak, and no new peaks appeared. This indicated that the AZ addition ratio did not cause a chemical reaction in the nanofiber membranes.

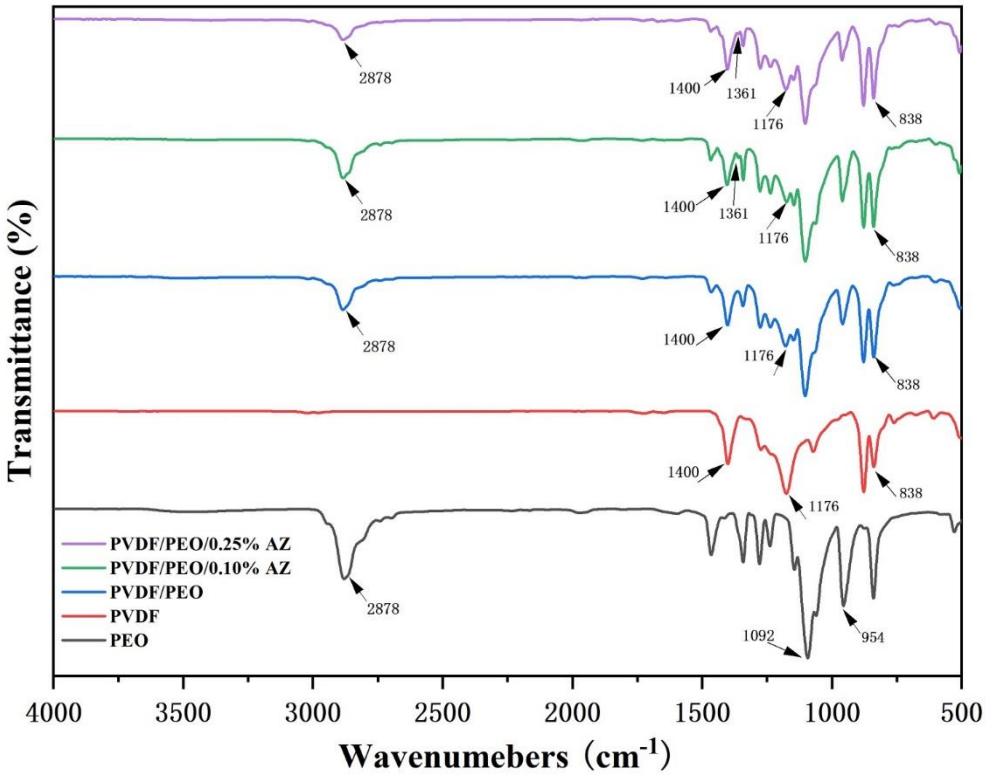

Figure 3. Infrared spectra of PEO, PVDF, PVDF/PEO, PVDF/PEO/0.1%AZ, and PVDF/PEO/0.25%AZ membrane

3.3. Contact angle of the nanofiber membranes

Dynamic contact angle experiments were performed on PVDF, PVDF/PEO, PVDF/PEO/0.1%AZ, and PVDF/PEO/0.25%AZ membranes (Figure 4). After the addition of hydrophilic PEO to PVDF, the contact angle of the PVDF/PEO membrane significantly decreased from 131.1° to 48.3° at 0 ms, indicating the transformation of a hydrophobic PVDF membrane into a hydrophilic PVDF/PEO membrane. The hydrophilicity of dressings effectively adsorbs wound exudates [26], so adding PEO to PVDF enhanced its performance as a wound dressing. After adding azithromycin, the contact angle increased from 48.3° to 64.6° at 0 ms due to its hydrophobicity, indicating decreased hydrophilicity of the PVDF/PEO/AZ membrane. Additionally, the contact angle of PVDF/PEO/0.25%AZ was larger than that of PVDF/PEO/0.1%AZ at 0 ms, indicating that more hydrophobic AZ further reduces the hydrophilicity of PVDF/PEO/AZ membranes. However, the contact angle of PVDF/PEO/0.25%AZ membranes was less than 90° at 500 ms, 800 ms, and 1000 ms, indicating the surface still exhibits hydrophilicity [27], and liquids were more likely to wet the membranes.

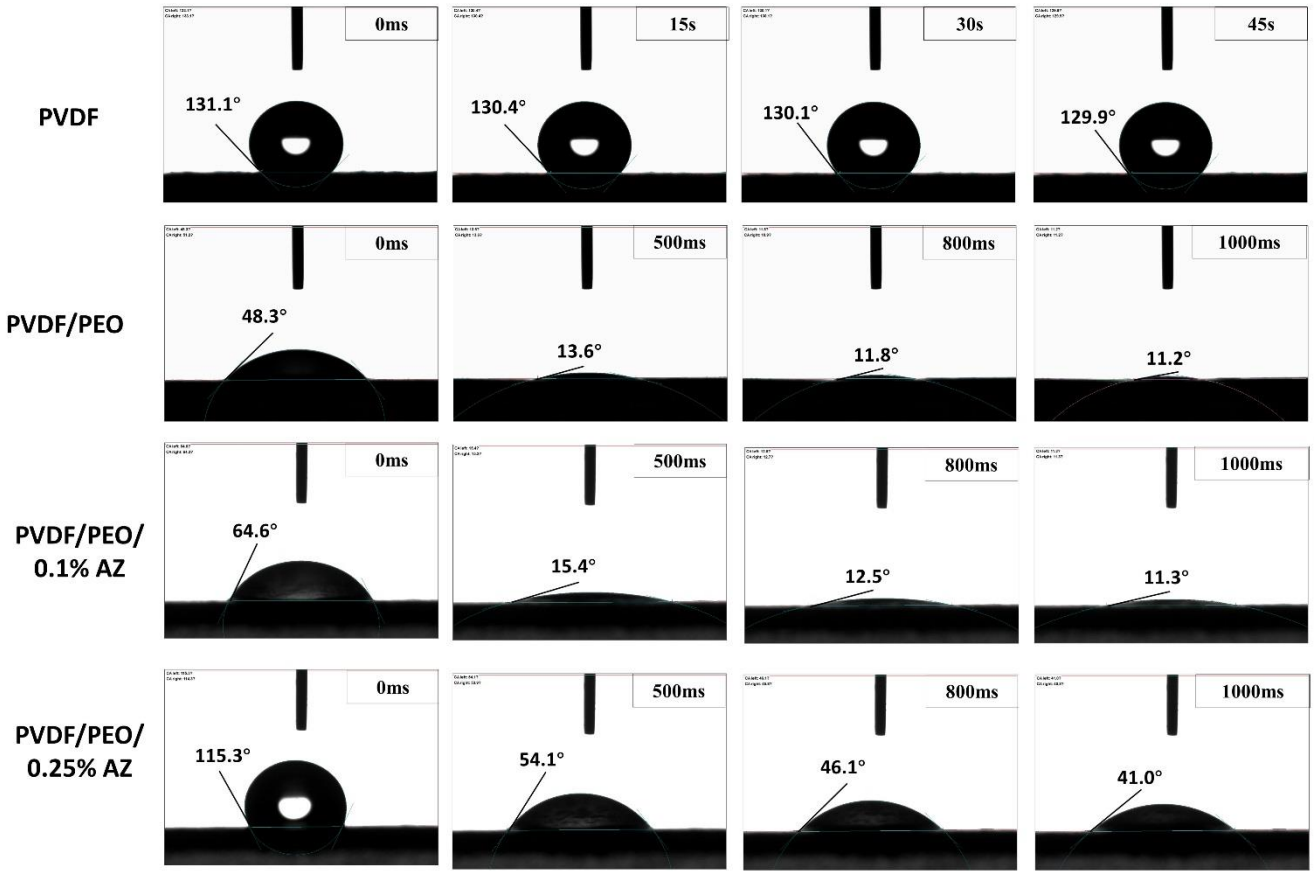

Figure 4. Dynamic contact angles of PVDF, PVDF/PEO, PVDF/PEO/0.1% AZ, PVDF/PEO/0.25% AZ membrane

3.4. Antibacterial experiment

To investigate the antibacterial effect of azithromycin-loaded fiber membranes, two Gram-positive bacteria (*S. aureus*, *B. subtilis*) and one Gram-negative bacteria (*E. coli*) were selected. According to the antibacterial experiment (Figure 5), the PVDF/PEO nanofiber membrane has nearly no antibacterial effect, while the PVDF/PEO/AZ membrane showed antibacterial circles with a diameter greater than 5 mm for *S. aureus*, *B. subtilis* and *E. coli*. Compared with the PVDF/PEO/0.1%AZ membrane, the PVDF/PEO/0.25%AZ membrane exhibited a larger diameter antibacterial zone, indicating superior antibacterial efficacy. The variation in sizes of antibacterial zones observed on the PVDF/PEO/AZ nanofiber membrane indicated that the antibacterial activity was dependent on the specific bacteria being tested. Comparing the antibacterial effect of AZ solution itself, the nanofibers loaded with AZ also had antibacterial effects on some Gram positive and Gram negative bacterias.

This suggested that the PVDF/PEO/AZ membrane may have different levels of effectiveness against different types of bacteria, potentially due to variations in their cell wall structures or susceptibility to the antimicrobial agents present in the membrane. Further studies and characterization are needed to understand the underlying mechanisms and optimize the membrane's antimicrobial properties for specific applications.

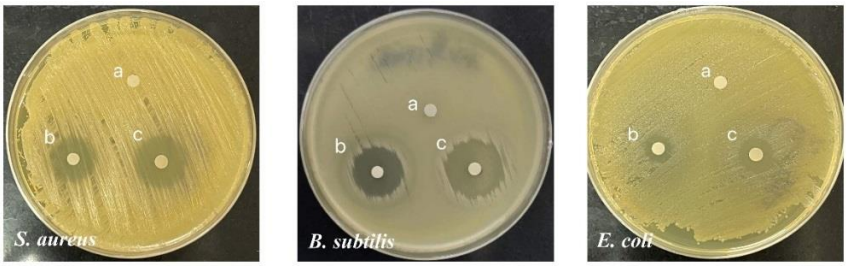

Figure 5. Antibacterial effects of PVDF/PEO membrane (a), PVDF/PEO/0.1% AZ membrane (b), PVDF/PEO/0.25% AZ membrane (c)

3.5. Animal experiments

Suppressing bacterial growth during wound healing is crucial in clinical practice [27, 28]， *S. aureus* is a major pathogen responsible for numerous wound infections [29, 30]. Therefore, the effects of PVDF/PEO/AZ was investigated in a full-thickness skin defect model infected with *S. aureus* (Figure 6). Two days after infection induction, all four wound groups exhibited varying degrees of infection. The control and PVDF/PEO groups exhibited yellow exudate, localized lesions with redness and swelling surrounding the infection. However, AZ loaded treatment alleviated the inflammatory response in PVDF/PEO/0.1%AZ and PVDF/PEO/0.25%AZ groups, reduced exudate significantly. Furthermore, compared to the control group, the PVDF/PEO group exhibited less yellow exudate, suggesting that PVDF/PEO contributes to reduced exudate formation. This was attributed to the strong hydrophilicity of the PVDF/PEO fiber membrane, enabling it to absorb wound exudate. However, there was no significant difference in wound healing between the PVDF/PEO/0.1%AZ and PVDF/PEO/0.25%AZ fiber membrane groups, and no statistically significant findings were observed.

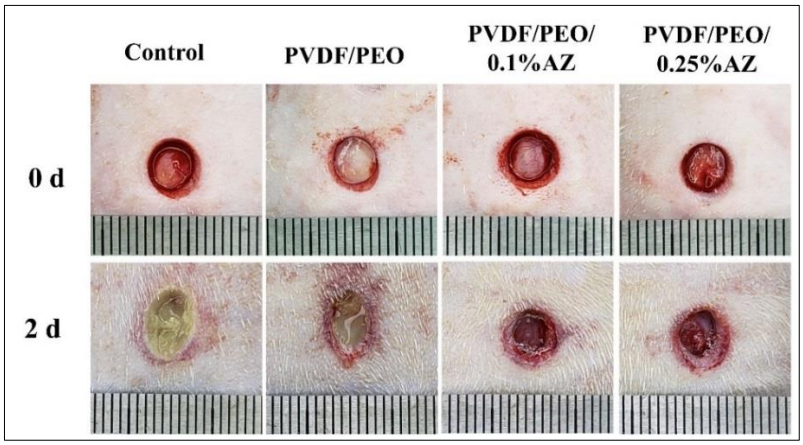

Figure 6. Wound infection situation in control and different Nanofiber membranes groups at day 0 and day 2

4. Conclusions

We successfully synthesized a novel PVDF/PEO/AZ nanofiber membrane containing antibacterial agent AZ using the electrospinning technique. The PVDF/PEO/AZ nanofiber membrane had uniform diameter and higher hydrophilicity than PVDF membranes. Antibacterial tests demonstrated that the PVDF/PEO/AZ membrane displayed antimicrobial activity against *S. aureus*, *B. subtilis* and *E. coli*, underscoring its potential as an effective antibacterial material. Furthermore, the PVDF/PEO/AZ nanofiber membranes could effectively prevent suppuration formation in full-thickness wound infected with *S. aureus.* These suggested that the PVDF/PEO/AZ membrane holds promise for preventing and treating bacterial infections in wounds. Future research will explore the potential of the PVDF/PEO/AZ membrane in enhancing skin wound healing. Furthermore, the study will investigate the antibacterial properties and effects on skin wound healing by incorporating other antibacterial components into PVDF/PEO nanofiber membranes.

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