WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 8, 646-661. Research Article ISSN 2277–7105

DEVELOPMENT OF ELECTROSPUN PVA- GELATIN/ZNO NANOFIBROUS FOR ANTIBACTERIAL WOUND HEALING APPLICATION

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Article Received on 04 March 2026,
Article Revised on 15 March 2026,
Article Published on 16 April 2026

https://doi.org/10.5281/zenodo.19592984

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How to cite this Article: Sathiya Nathan S.[1], Nanthakumar G.[2], Vijaykumar A.[1], Sanjai Gandhi K.[2], Amutha R.*[1] (2026). Development Of Electrospun Pva- Gelatin/Zno Nanofibrous For Antibacterial Wound Healing Application. World Journal of Pharmaceutical Research, 15(8), 646–661.

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ABSTRACT

The development of advanced wound dressings that promote rapid healing and prevent infection is a growing focus in biomedical research. In this study, a biocompatible composite nanofibrous composed of polyvinyl alcohol (PVA) and gelatin, incorporated with zinc oxide (ZnO) nanoparticles, was fabricated for potential use in wound healing applications. PVA provides excellent fiber forming properties and mechanical strength, while gelatin enhances biocompatibility and cell adhesion. ZnO nanoparticles were synthesized and integrated into the polymer matrix to impart antibacterial activity and promote tissue regeneration. The composite nanofibers were characterized using FTIR, SEM, XRD, and mechanical testing to assess structural integrity and nanoparticle dispersion. In vitro antibacterial studies demonstrated significant activity against both Gram-positive and Gram-negative bacteria, while biocompatibility tests using fibroblast cells confirmed non-cytotoxic behavior. The wound healing potential of the nanocomposite was evaluated enhanced cell migration and proliferation. The synergistic properties of PVA, gelatin, and ZnO nanoparticles suggest that the developed nanocomposite holds great promise as an effective wound dressing material.

KEYWORDS: PVA, Gelatin, Zinc oxide nanoparticles, Electrospinning, SEM, FTIR, XRD, Antibacterial activity, Wound healings.

1. INTRODUCTION

Nanotechnology is the area of science and engineering that focuses on creating and using structure, devices and system that have one or more dimensions on the order of 100 nanometer (100 millionth of millimetre) or less by manipulating atoms and molecules at the nanoscale.[1] Although there are several examples of structures with one or more nanometer dimensions in the natural world and many technologies have unintentionally used them for a long time, it has only lately become feasible to do so on purpose. Numerous uses for nanotechnology, involve novel materials with entirely unique characteristics and outcomes when compared to the identical materials produced at bigger scale.[2] This is caused by effects that are visible at that small size but are not visible at bigger scale, as well as the extremely high surface to volume ratio of nanoparticles compared to larger particles. Nanotechnology applications have the potential to be very helpful and have a big impact on society. The skin is the largest organ of the human body that plays a crucial role in protecting the underlying organs from pathogens and microorganisms. During the skin injury, microorganisms can infect the wounded bed and may cause septicaemia or chronic infection if the infiltration of the organisms continues.[3] Antibiotic resistance is the main reason of chronic infections, which may lead to difficulties in treatment and delays the healing process. Furthermore, the presence of multidrug-resistant microorganisms has presented a fundamental challenge due to the reduction of antibiotic treatment options. It is predicted by the Centre for Disease Control and Prevention (CDC) that antimicrobial-resistant could lead over 10 million deaths a year up to 2050.[4]

On the other hand, the wound dressing itself could be the source of contamination due to the wound bandage – discharges interfaces which become a medium for growth of microorganisms. Another problem is that the traditional wound dressings need to be replaced regularly and often adhere to the wound site, which can often lead to secondary tissue injury. One of the most important aspects for clinical success of wound healing is the use of biocompatible and biodegradable wound dressing materials so that the additional surgical step for removing the dressing from the regenerated site would be eliminated.[5] In recent years the production of electrospun wound dressings with bactericidal activity has attracted increased attention. Dressings produced by electrospinning technique possess high porosity

levels with high surface area to volume ratio. Electrospun structures contain nano to micro size fibers that resemble the morphological properties of extracellular matrix (ECM). These properties make the electrospun membranes suitable for tissue engineering applications.[6]

The information and communications industries, as well as the food and energy industries, have already embraced nanotechnology. It is also employed in several medical products and medications. Nanomaterials might also present fresh possibilities for lowering environmental pollution. Nanofibers are fibres with a diameter between one nanometer and one micrometre, generally.[7] Nanofibers can be produced from a variety of polymers, giving them a range of physical characteristics and possible uses. More than four centuries ago, electrospinning was used to create the first nanofibers. Applications for nanofibers include cancer diagnostics, medication delivery, tissue engineering, optical sensors, air filtration, oil water separation and sportswear textile, lithium air batteries. In this study, both natural and synthetic polymers are used. They are polyvinyl alcohol, gelatin and loaded with zinc oxide nanoparticles.

1.1 Polyvinyl alcohol (PVA)

PVA is a biocompatible polymer with great mechanical stability and flexibility that can be blended with gelatin in order to improve its low mechanical properties. Unlike other synthetic polymers, PVA is soluble in water without need to use any other chemical solvents. Non-toxicity, chemical and thermal stability are other positive characteristics of PVA.[8] However, in comparison to gelatin, it lacks the cellular recognition sites and thus its bioactivity is limited. Poly (vinyl alcohol) (PVA) is an odourless and tasteless, translucent, white or cream-colored granular powder, which is discovered by *Herrmann* and *Haehnel* in 1928. PVA is a water-soluble, nontoxic, semi crystalline, biocompatible, and biodegradable polymer. It is widely utilized for various applications such as textile sizing, paper coating, flexible water-soluble packaging films, controlled drug delivery systems, dialysis membrane, wound dressing, and artificial skin.[9] This broad range of applications is due to its outstanding properties such as high oxygen and aroma barrier properties, high tensile strength and flexibility, excellent film forming, emulsifying, and adhesive properties. The melting point of PVA is 230°C and 180–190°C for the fully hydrolysed and partially hydrolysed grades, respectively. Its decomposition temperature is above 200°C and it can undergo pyrolysis at high temperatures. The vinyl alcohol tautomerizes to acetaldehyde, which is more stable than vinyl alcohol at room temperature. Thus, PVA is not built up in polymerization reactions from vinyl alcohol monomer. The basic monomer for PVA is vinyl acetate (VA). This

polymer is prepared by the polymerization of vinyl acetate to poly (vinyl acetate) (PVAc), followed by hydrolysis of PVAc to PVA with the use of either acid or base as a catalyst. The sequence of reactions is show in Fig.1

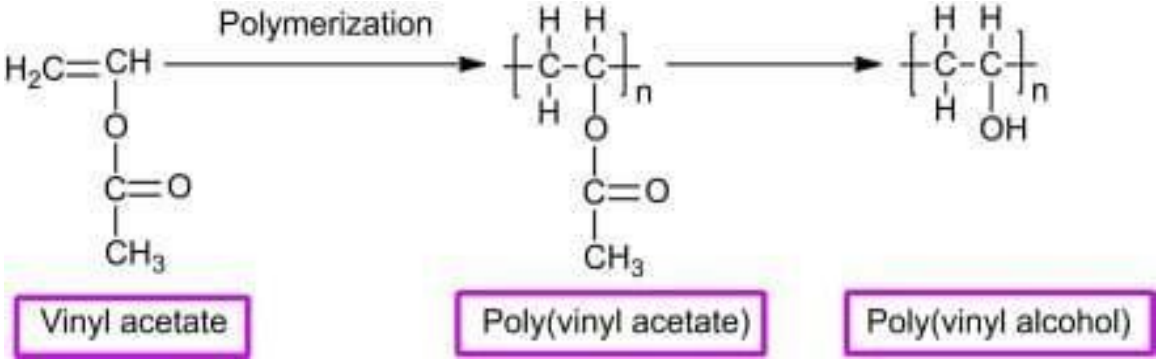

Fig: 1 Chemical structure of poly vinyl alcohol [PVA]

1.2 Gelatin

Gelatin is also a natural polymer derived from collagen of animal skin and bones. The molecular weight approximately 50–100 kDa. It is translucent, colourless, brittle and tasteless. It is biodegradable in nature.[10] Fig 2 represented the chemical structure of gelatin. It has good film forming property and known for its wound healing properties by preventing fluid loss due to exudation. It is a good source of protein and promotes general joint health and stiffness in athletes. Gelatin is a water soluble polymer and is highly noteworthy in the field of tissue regeneration, as it possesses amino and carboxyl functional groups of collagen and provides cellular affinity for adhesion and proliferation throughout the scaffold. It also provides a moist condition over the wound site for better wound healing. Nevertheless, weak mechanical strength and rapid degradation rate of gelatin-based scaffolds make this polymer less applicable for biomedical usages. Gelatin comprises of proline ($C_2H_5NO_2$), glycine ($C_5H_9NO_2$) and hydroxyproline ($C_5H_9NO_3$) and is similar in the composition of amino acids as Collagen, mimicking the extracellular matrix.[11] The composition of glycine amino acid in the gelatin is responsible for the adherence of cells. The structure of gelatin mainly depends on the extraction process. Based on the process of extraction, gelatin can be categorized into two types, namely, type A and type B. Type A (positively charged) refers to acid extraction, while type B (Negatively charged) refers to alkaline extraction.

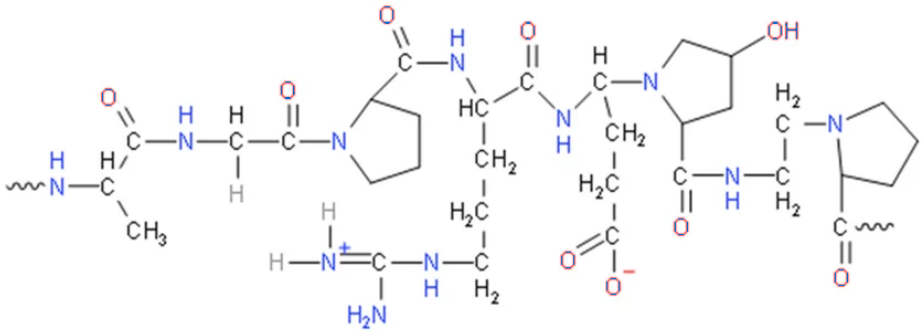

Fig 2: Chemical structure of Gelatin.

1.3 Zinc oxide Nanoparticles

Zinc oxide nanoparticle is among one of the most researched studies conducted due to its ability to apply in varied downstream applications. Zinc oxide nanoparticle is the second most abundant metal oxide after iron and it is inexpensive, safe, and as well as it can be prepared easily.[12] Physical and chemical behaviors of zinc oxide nanoparticle can be easily turned by changing the morphology by using different synthesis routes or different precursors or different materials to produce the nanomaterial. Zinc oxide nanoparticle is one of the inorganic compounds of group II–IV semiconductor for analytical sensing applications. Zinc oxide nanoparticle appears to be white powder and insoluble in water.[13] Zinc and its oxide are among the metals with biological effects that have been extensively investigated. Zinc is an active element with significant chemical properties. The functionalization of ZnO molecules by bioactive compounds can produce ZnO nanoscale materials with better activity. It is possible to create ZnO nanoparticles by biological, chemical, or physical processes. The synthesis method determines the nanoparticle's crystal formation, shape, size, size distribution, stability, and aggregation characteristics. The most potent microbial killers are nanoparticles made of metals and their oxides, such as Zn, Ag, etc. Bacterial infections are considered serious health issues all over the world. The rise in pathogenic strain outbreaks, novel bacterial mutations, and antibiotic resistance have all contributed to the need for the development of more potent antibacterial drugs.[14] ZnO nanoparticles are widely recognized to have antibacterial attributes, with activities directly correlated to their concentration, and inversely correlated to their particle size.

1.4 Electrospinning

Electrospinning is a widely used technique for nanofiber synthesis. Electrospinning proves to be an efficient technique for producing nanofibers, typically with average diameters spanning from tens to hundreds of nanometers. Electrospinning is carried out using polymeric

materials to draw continuous fibers under a high-voltage electric field.[15] A traditional electrospinning setup consists of four main components: a high-voltage source, a needle or spinneret, a syringe pump, and a collector. The polymeric solution or polymer melt is transferred to the syringe and subsequently linked to the syringe pump. When a high voltage is applied to the pendant droplet formed when the liquid is extruded from the spinneret, the droplet will start to elongate into a conical shape known as a Taylor cone.[16] This leads to the formation of a liquid jet that is directed towards the collector. As the liquid jet makes its way to the collector, the solvent existing in the polymer solution will evaporate, leading to the creation of solid fibers. On the other hand, the solid fibers will take shape as the polymer cools down. This is the basic principle behind electrospinning. One of the major advantages of electrospinning is the ability to use many different types of materials such as organic materials, inorganic materials, and even organic and inorganic hybrids.[17] However conventional electrospinning has certain limitations such as low throughput. Certain materials with low solubility in various solvents or high electrical resistivity are not suitable for efficient use. Therefore, considerable amounts of research were done to develop more advanced spinning system.

1.5 Wound Healing

The biological process is categorised into four continuous and overlapping phases haemostasis, inflammation, proliferation, and remodelling, which results in tissue restoration. Skin is the most commonly and widely affected organ in case of injury or wounds. Depending on the extent of the injury, skin wounds are the result of degradation of the epidermal, dermal and hypodermal layers of the skin.[18] Acute wounds follow a structured wound healing process, usually regaining skin integrity in 4–12 weeks. In contrast, chronic wounds take longer to heal more than 12 weeks. This may increase the chances of infection.[19] The optimal healing process involves rapid haemostasis, inflammation; mesenchymal cell differentiation, proliferation, and migration and angiogenesis, re-epithelialization; and synthesis, cross-linking, and alignment of collagen to provide structural and mechanical strength to the healing tissue.

2. MATERIALS AND METHODS

2.1 Materials

Poly vinyl alcohol (PVA) with molecular weight (1,25, 000 kDa). It depends powder in from and purchased from OTTO-chemika-biochemika-reagents. Gelatin [Gel, from bovine skin,

Type B], molecular weight (477.550 kDa) purchased from Sigma-Aldrich. Zinc acetate, sodium chloride and Distilled water.

2.2 Synthesis of Zinc Oxide Nanoparticles

A total of 4 g of zinc acetate was dissolved in 100 mL of distilled water in a beaker, and the solution was stirred using a magnetic stirrer on a hot plate at 30 °C for 40 minutes. Subsequently, 0.2 M NaOH was added dropwise to the solution under continuous stirring until the pH reached 11.0. The solution turned white due to the formation of a precipitate, and stirring was continued for 1 hour, followed by allowing the mixture to stand for 50 minutes.[20] The supernatant was then decanted, and the synthesized precipitate was washed repeatedly with distilled water until neutral pH was achieved, followed by a final wash with ethanol. Finally, the obtained precipitate was dried in a hot air oven at 60–70 °C for 5 hours to facilitate the decomposition of $Zn(OH)_2$ into ZnO nanoparticles.

2.3 Preparation of Polymer Solution

A 10% [w/v] polyvinyl alcohol and gelatin solution was prepared by dissolving the polymers in distilled water and stirring using a magnetic stirrer for approximately 2 hours until a homogeneous solution was obtained. Zinc oxide nanoparticles [0.5–1%] were then incorporated into the polyvinyl alcohol–gelatin solution and stirred continuously using a magnetic stirrer to ensure even distribution.

2.4 Electrospinning Process

The electrospinning from the mixed solutions was carried out at room temperature. The experimental setup used for electrospinning consisted of a syringe pump, on which a 2ml syringe was connected to the stainless steel needle, a high voltage power supply which generated DC voltage in a range of 0-50 kV and a grounded plate receiver covered with aluminium foil. The pumping speed was set at 0.5 ml/h, and applied voltage was set at 27 kV. The late was placed 15cm from the tip of the nozzle and used to collect the as electro-spun nanofibers. The electrospinning setup and schematic diagram of the electrospinning process are shown in Figure 3.

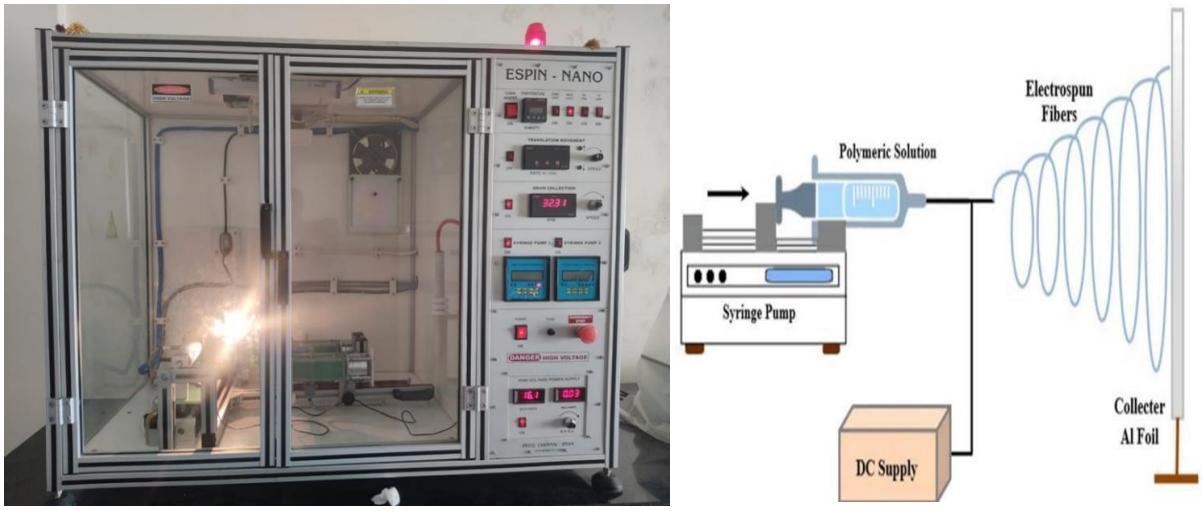

Fig: 3 Electrospinning apparatus and schematic representation of the electrospinning process.

2.5 Scanning Electron Microscope (SEM)

The morphology of the electro spun nanofibers was observed with a scanning electron microscope under high vacuum. The SEM combines high contrast imaging, broad depth of field and high spatial resolution to examine and characterize features at the nanofiber scale. The accelerating voltage of 10-15kV. The diameter of nanofibers was measured by using image analyser. The diameters of fibres were measured randomly on 30-60 fibres of sample using the Nano measure 1.2 software and they provided the average diameter as well as the size distribution of the fibres.

2.6 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an analytical technique used to identify their functional groups and incorporation of nanoparticles in organic, polymeric and in some cases inorganic materials. The FTIR analysis method uses infrared light to scan test sample and observe chemical properties. PVA and Gelatin/ZnO nanoparticles were analysed by Fourier Transform Infrared Spectroscopy [FTIR] in a scanning range 400-4000 cm-1.

2.7 X-RAY Diffraction

XRD analysis was performed to study the crystallographic structure of synthesis of ZnO nanoparticles and to confirm their PVA and gelatin polymer matrix. The intensity of these ZnO-related peaks increased with higher nanoparticle loading, demonstrating the dose-dependent crystallinity. The coexistence of amorphous polymer peaks and crystalline ZnO

peaks indicates the formation of a semi-crystalline nanocomposite, which provides a good balance of mechanical strength and flexibility ideal for wound dressing applications.

2.8 Antibacterial Activity

Well diffusion methods against Staphylococcus aureus and Escherichia coli demonstrated significant antibacterial activity in ZnO-loaded fibers. The size of inhibition zones increased with ZnO concentration, proving its dose-dependent antibacterial efficacy.[21] The assay was performed on Muller Hinton Agar (MHA) plates to ensure optimal bacterial growth and clear zone visualization. The plates were incubated at 37˚C for 24hrs after which the zone of inhibition around the wells were measured by millimetre [mm].

2.9 MTT Assay

The cytocompatibility of the fabricated nanocomposite fibers was evaluated using the MTT assay with L929 fibroblast cells. The cells were seeded in a 96-well plate at an appropriate density and incubated for 24 hours to allow cell attachment. After incubation, the cells were treated with nanocomposite fiber extracts prepared in culture medium and further incubated for 24 hours. Subsequently, MTT reagent was added to each well and incubated for 4 hours at 37°C. The formed formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm using a microplate reader to determine cell viability.

3. RESULTS

3.1 SEM Analysis of Scaffolds

Figures 1-5 show the SEM images of electrospun nanofibers composed of polyvinyl alcohol (PVA), gelatin (GE) and zinc oxide (ZnO) nanoparticles at 8% concentration. The electrospinning parameters were maintained at a tip-to-collector distance (TCD) of 10 cm and an applied voltage of 22 kV. The resulting nanofibers exhibit a uniform, bead-free morphology with interconnected networks. The average fiber diameters varied depending on the PVA/GE composition and presence of ZnO nanoparticles. It was observed that increasing the PVA content led to thicker fiber diameters, while higher gelatin content enhanced the mechanical properties, producing thinner fibers with improved tensile strength. Incorporation of ZnO nanoparticles at 8% concentration resulted in a slight reduction in fiber diameter and improved uniformity due to increased solution conductivity. The optimal morphology was achieved at 22 kV, which produced consistent nanofibers with minimal defects. At this voltage and TCD, the average fiber diameter ranged from approximately 80 nm to 350 nm, indicating a well formed nanofibrous mat suitable for antibacterial and protective

applications. These findings suggest that ZnO nanoparticles not only contribute to antimicrobial functionality but also influence the fiber formation process during electrospinning, enhancing the overall structural integrity and uniformity of the PVA/GE blend.

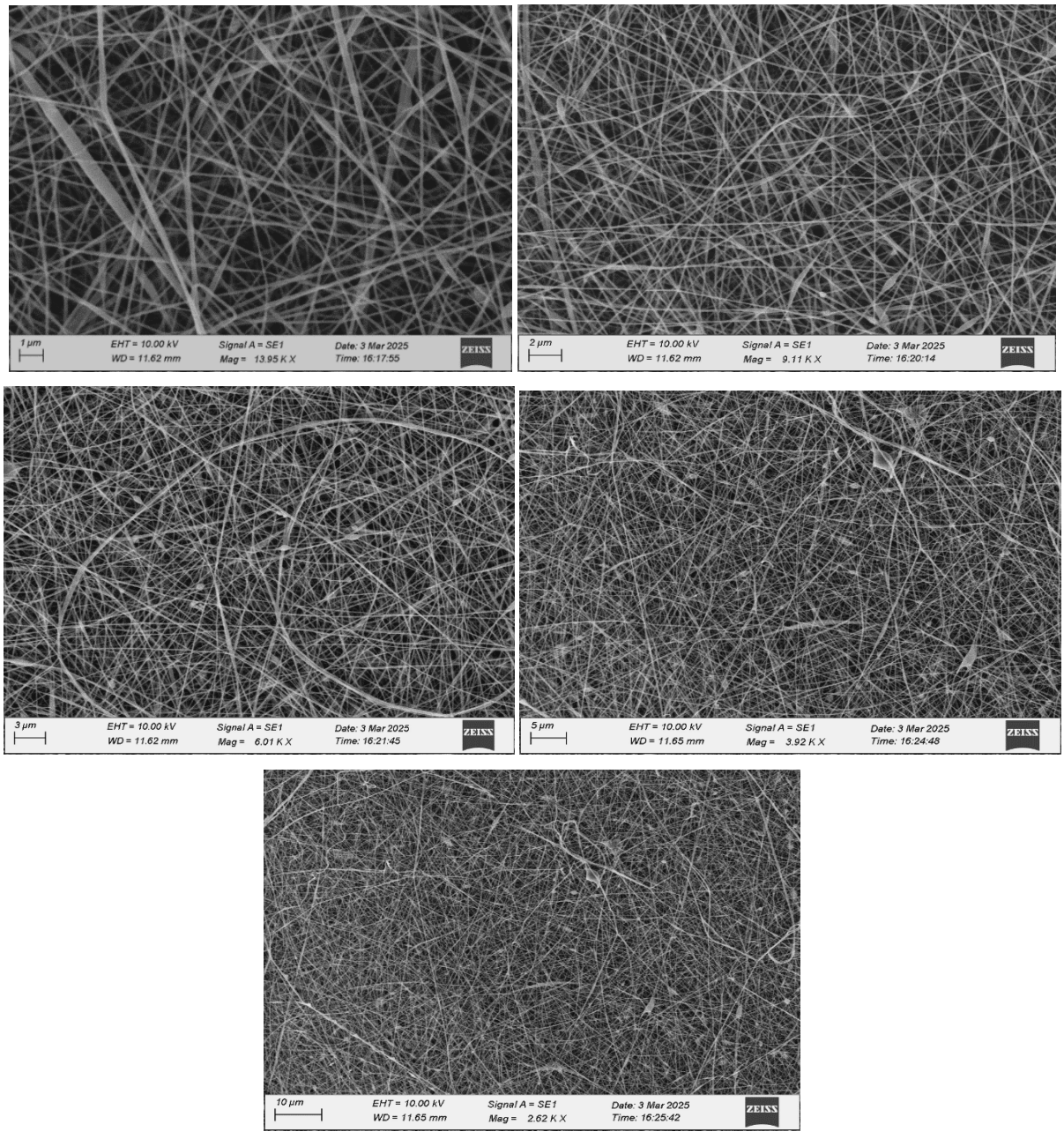

Fig: 4 SEM images of electrospun PVA–gelatin/ZnO nanofibers.

3.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The spectra of the PVA [Poly vinyl alcohol], Gelatin, Zinc oxide nanoparticles and their composites membrane were analysed by Fourier transform infrared spectroscopy in scanning range of 500-4000 cm-1 for spectral resolutions of 4cm-1. The FTIR spectra were recorded to

identify functional groups and interactions between the polymer matrix and ZnO nanoparticles, as shown in Figure 5. The FTIR spectra gave information about structure and interaction of the blended membranes studied. The spectra of PVA, Gelatin and Zinc oxide.

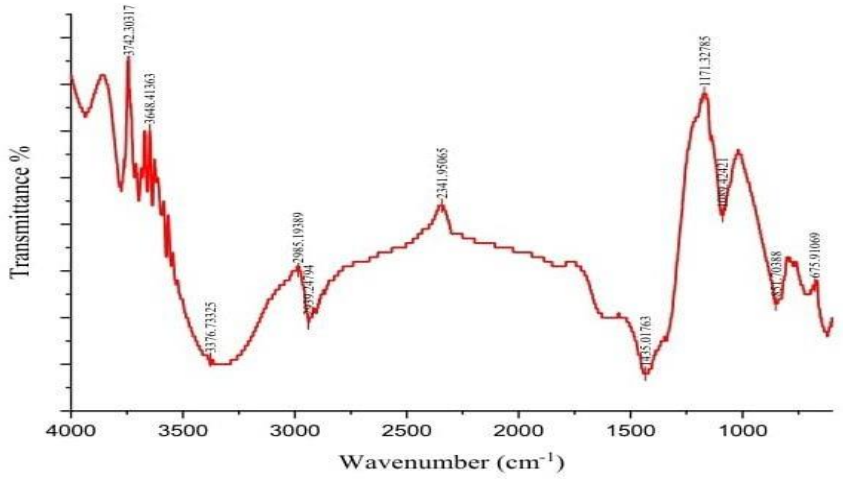

Fig 5: FTIR analysis of nanofibers.

nanoparticles blended membranes in the range of 4000-600 cm-1. The PVA strongest absorption peak indicating at 1090-1140 cm-1 at presence of (c-o stretching), Gelatin strongest absorption peak is indicating at 1650 cm-1 at presence of (c=o stretching) and zinc oxide nanoparticles strongest absorption peak is indicating at 650-750 cm-1 (Zn-o stretching).

3.3 X RAY DIFFRACTION

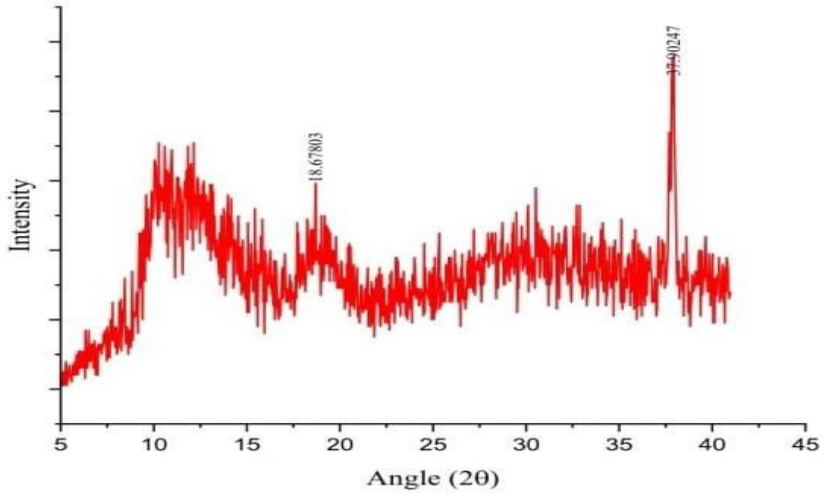

Fig: 6 X**-ray diffraction [XRD] pattern of the prepared sample showing diffraction peaks at different 2θ angles**.

The XRD pattern shows a broad peak at 18.67° and a sharp peak at 36.02°, indicating a partially crystalline structure. The broad hump suggests an amorphous or polymeric matrix,

while the peak at 36.02° corresponds to the (101) plane of ZnO, confirming the presence of ZnO nanoparticles. The overall pattern indicates Nano crystalline ZnO embedded in an amorphous or low-crystalline phase, suitable for wound healing applications. XRD pattern is shown in Figure 6.

3.4 Antibacterial Activity

The antibacterial activity of the PVA/gelatin/ZnO nanocomposite increased with increasing concentration against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. The nanocomposite showed a zone of inhibition ranging from 3 mm to 8 mm against *E. coli* and 2 mm to 6 mm against *S. aureus*. The highest antibacterial activity was observed at 100 µL concentration, showing 8 mm inhibition zone for *E. coli* and 6 mm for *S. aureus*. The results indicate that the ZnO-incorporated nanocomposite exhibited stronger antibacterial activity against Gram-negative bacteria compared to Gram-positive bacteria, which may be attributed to differences in cell wall structure and ZnO nanoparticle interaction with bacterial membranes.

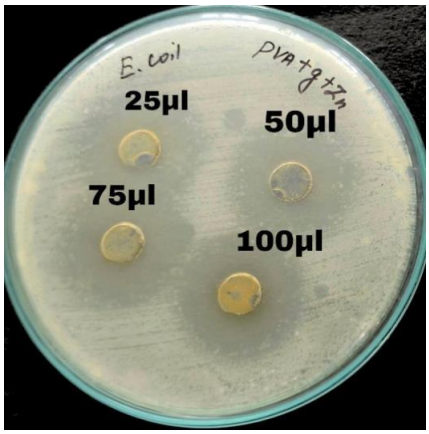

Fig: 7 *E.Coil* [PVA /gelatin and ZnO]

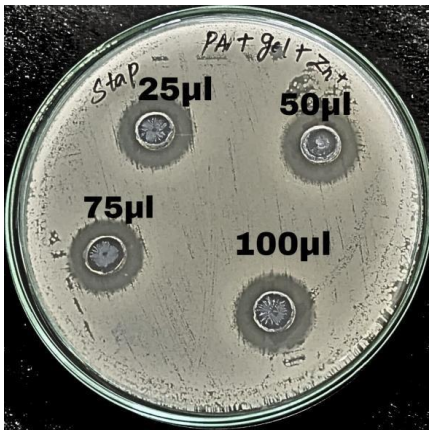

Fig: 8 *Staphylococcus aureus* [PVA /gelatin and ZnO]

Table 1: Zone of Inhibition of PVA/Gelatin/ZnO Nanocomposite.

Test Organisms	Volume (PVA/Gelatin/ ZnO)	Zone of Inhibition
Escherichia Coli	25	3mm
	50	4mm
	75	6mm
	100	8mm
Staphylococcus aureus	25	2mm
	50	3mm
	75	4mm
	100	6mm

3.5 MTT Assay

The cell viability results demonstrated that the percentage of cell growth remained above 90% at all tested concentrations (12.5–200 µg/ml), indicating that the prepared material exhibits low cytotoxicity and good biocompatibility. A slight decrease in cell viability was observed with increasing concentration; however, the overall cell growth remained high, confirming that the material is safe for biomedical applications.

Table 2: Absorbance values and calculated cell viability (%) of L929 fibroblast cells treated with different concentrations of the sample material.

Conc	12.5µg	25µg	50µg	100µg	200µg	Cont
ABS	0.556	0.545	0.541	0.534	0.524	0.567
	0.554	0.547	0.541	0.533	0.525	0.563
	0.552	0.548	0.542	0.536	0.526	0.565
Avg	0.554	0.54666667	0.54133333	0.53433333	0.525	0.565

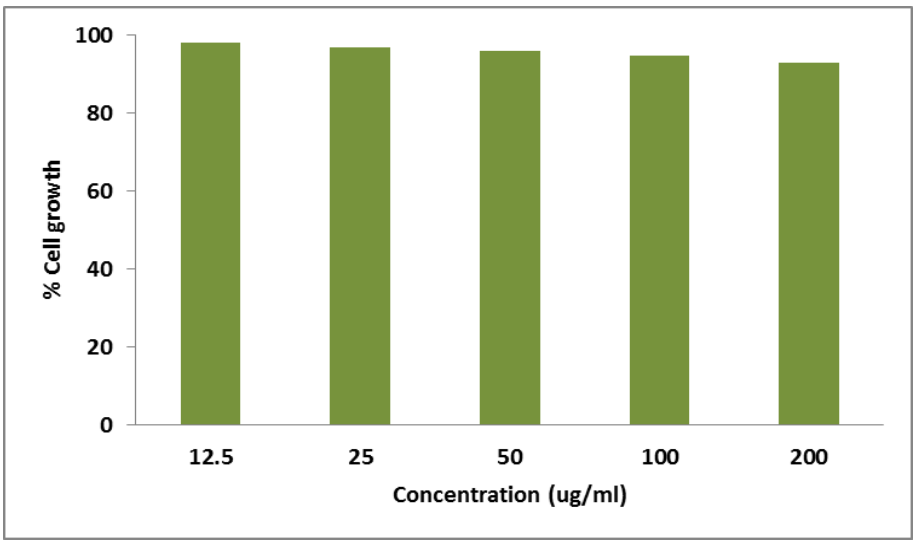

Fig 9: Cell viability (%) of L929 fibroblast cells at different concentrations (12.5- 200 µg/ml).

4. CONCLUSION

The fabricated PVA/gelatin/ZnO nanocomposite fiber exhibited promising characteristics for wound healing applications. The blend of PVA and gelatin resulted in flexible and biocompatible fibers, while the incorporation of ZnO nanoparticles provided additional benefits such as antibacterial activity and enhanced structural properties.[22] Characterization studies confirmed the successful integration of ZnO nanoparticles. FTIR analysis showed no significant chemical incompatibilities between the components. XRD analysis indicated the presence of crystalline ZnO within the semi-crystalline polymer matrix. SEM images

revealed uniform dispersion of nanoparticles, contributing to surface roughness favourable for cell attachment. Mechanical testing demonstrated that ZnO improved tensile strength up to an optimal concentration. Biological evaluations further supported the nanocomposite's effectiveness. The films exhibited strong antibacterial activity against both *E. coli* and *S. aureus*, suggesting effective infection control. Biocompatibility tests (MTT assay) confirmed that the films are non-toxic to fibroblast cells. A PVA/gelatin/ZnO nanocomposite fiber was successfully fabricated using a simple and reproducible method. ZnO nanoparticles were effectively synthesized and integrated into the polymer matrix, imparting both antibacterial and mechanical reinforcement properties. The fiber showed excellent biocompatibility, antimicrobial performance, and wound healing potential, making them ideal candidates for next-generation wound dressings. The combination of natural [gelatin] and synthetic (PVA) polymers, reinforced with inorganic ZnO nanoparticles, resulted in a multifunctional material with desirable biomedical properties.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: No funding was obtained to assist in the development of this manuscript.

Conflicts of Interest: The author declares no conflict of interest.

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