

RESEARCH ARTICLE OPEN ACCESS

Optimization of the Electrospinning Process to Create an Active Bilayer Nanostructured Wound Dressing Based of PLA and Ibuprofen

Anne-Sophie Schuller^{1,2}  | Élise Girault^{2,3}  | Amdouni Sonia^{1,2,3} | Leonard Ionut Atanase^{4,5}  | Cristina Elena Horhoge⁶  | Floriane Leclinche^{2,3}  | Christelle Delaite^{1,2} 

¹Laboratoire de Photochimie et d'Ingénierie Macromoléculaires (LPIM), Université de Haute Alsace (UHA), Mulhouse, France | ²Université de Strasbourg, Strasbourg, France | ³Laboratoire de Physique et Mécanique Textiles (LPMT), Université de Haute Alsace (UHA), Mulhouse, France | ⁴Academician Ioan Hăulică Research Institute, Faculty of Medicine, Apollonia University of Iași, Iași, Romania | ⁵Academy of Romanian Scientists, Bucharest, Romania | ⁶Department of Public Health, Faculty of Veterinary Medicine, Ion Ionescu de la Brad, Iasi University of Life Sciences, Iasi, Romania

Correspondence: Anne-Sophie Schuller (anne-sophie.schuller@uha.fr)

Received: 22 May 2025 | **Revised:** 18 July 2025 | **Accepted:** 19 July 2025

Funding: The authors received no specific funding for this work.

Keywords: (wound) dressing | electrospinning | polylactic acid | zinc oxide

ABSTRACT

A new bilayer wound dressing structure has been developed by depositing amorphous polylactic acid (PLA) nanofibers on a hydrogel layer composed of gelatin (GE), hyaluronic acid (HA) and zinc oxide (ZnO) particles for its antibacterial properties. The choice of solvent was crucial to optimize electrospinnability, productivity, and nanofibers morphology. This study investigated the impact of polymer concentration and electrospinning parameters, such as applied voltage and distance from tip to collector, on nanofibers morphology. To improve the healing process, Ibuprofen (IBU) was encapsulated in PLA nanofibers at a concentration of 5 wt% due to its anti-inflammatory properties. Differential scanning calorimeter (DSC) analysis confirmed the amorphous nature of PLA, while TGA confirmed the presence of IBU and almost complete evaporation of solvent during the electrospinning process. Scanning electron microscopy (SEM) characterization demonstrated that nanofibers fabricated from PLA/IBU solutions electrospun in acetone at concentrations of 10 wt% and 12.5 wt%, using an applied voltage of 40 kV and a tip-to-collector distance of 15 cm, yielded fibers with the most favorable morphological features for the target application. The resulting nanofibers exhibited consistent nanoscale diameters and a smooth, homogeneous surface topology, indicating that these electrospinning parameters represent optimal conditions for achieving high-quality fiber formation. Antimicrobial analyses were conducted to demonstrate the bilayer dressing's effectiveness in inhibiting or eliminating bacterial growth. These tests aimed to assess the potential of this new dressing as a protective barrier against infection, highlighting its relevance for wound care applications.

1 | Introduction

The skin is one of the body's most exposed and strained organs throughout the lifetime. It is a complex and versatile organ, the largest in the body, with an average surface area of 2 m². It is the first point of contact with the external environment and acts as a physical barrier [1]. However, despite

its remarkable capacity to regenerate, the skin is prone to various types of injuries, from minor scratches and cuts to more serious burns and wounds that can even be life-threatening. Many skin lesions can be treated by applying a wound dressing [2]. Conventional dressings help to manage wound healing by protecting them from the external environment while keeping them moist to accelerate healing. Ideally, the dressing

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Journal of Applied Polymer Science* published by Wiley Periodicals LLC.

should mimic the extracellular matrix (ECM) and promote cell adhesion and proliferation to accelerate skin regeneration. Thus, the ideal dressing should protect the wound from external contaminants and facilitate rapid healing [2]. Traditional monolayer dressings are unable to meet these requirements as they do not facilitate rapid wound healing; therefore, bilayer dressings have received particular attention in recent years. In fact, the two separate layers have different properties and offer versatile features such as improved exudate absorption, moisture management [3], and controlled drug delivery to relieve pain and prevent inflammation [4–6].

Electrospun bilayer dressings can be prepared by different electrospinning techniques such as single needle, multiple needles, and needleless process [7]. Electrospun nanofibers possess structural similarities to the fibrils in native ECM. They also present a high surface area for increasing cell-material adhesion and interaction, as well as adjustable chemical, physical, and biological properties for regulating cellular activities, remodeling ECM deposition, and facilitating tissue regeneration [1].

The use of a needle allows the formation of nanofibers but has many limitations, such as lower production, clogging of the needle, and significant loss of time. Conversely, needleless electrospinning, which is a semi-industrial or industrial technique, enables larger quantities to be produced rapidly: this allows the possibility to obtain a thicker membrane in a few minutes [8]. Numerous studies have been carried out on needleless electrospinning to overcome the disadvantages of electrospinning with needles. They have highlighted the application of this technique in the biomedical field as well as in tissue engineering. Because of its flexibility, needleless electrospinning can be adapted to a wider variety of fields, enabling the production of nanofibers with diameters ranging from a few tens of nanometers to several micrometers, while offering advantageous productivity [9–11].

In addition to the manufacturing process, the choice of materials is of importance in ensuring the performance and efficiency of the final dressing. Careful selection of the materials used in the design of dressings ensures improved functional properties and overall effectiveness [12, 13]. Many natural and synthetic polymers are commonly used for this purpose, such as gelatin (GE), a polymer known for its biocompatibility, biodegradability, and hydrophilic nature. Gelatin offers high mechanical properties and can be reshaped into a hydrogel, particularly suitable for applications requiring moisture and flexibility [12]. Hyaluronic acid (HA) is also a biological material of choice for wound healing, owing to its beneficial effects on tissue regeneration and remodeling of the ECM [14–17]. Moreover, HA accelerates healing while maintaining adequate hydration, essential for tissue regeneration. The addition of zinc oxide (ZnO) particles gives the dressing antimicrobial and anti-inflammatory properties. ZnO is an inert filler and is widely used for its ability to fight infection while promoting healing [18–22]. Finally, the use of polylactic acid (PLA), a biodegradable and biocompatible polymer derived from renewable resources, adds mechanical stability and controlled degradation to the dressing. The incorporation of ibuprofen (IBU), a non-steroidal anti-inflammatory drug, into PLA enables prolonged drug release, which enhances therapeutic outcomes while mitigating local inflammatory responses [12, 23, 24].

The main objective of this research was to develop an innovative bilayer dressing designed to provide controlled release of IBU in order to accelerate wound healing. The dressing consists of two distinct layers, each of which plays a crucial role in the healing process. The first layer, composed of a hydrogel based on gelatin, HA, and zinc oxide, was designed to maintain a moist environment around the wound, absorb exudate, and provide antibacterial properties to prevent infection. The second layer, which comes into direct contact with the skin and wound, was based on PLA nanofibers containing IBU, enabling a prolonged and controlled release of the drug. To validate the efficiency of this bilayer dressing, thermal and in vitro biological characterizations were carried out, providing an in-depth understanding of the material's performance under clinically relevant conditions.

2 | Materials and Methods

2.1 | Materials

PLA 4060D ($M_w=243,000$ g/mol) in round bead form was sourced from NatureWorks; Ibuprofen (IBU) in >98% pure form was supplied by Sigma-Aldrich; acetone (AC) in >99.8% purity was supplied by Carlo Erba; and N,N-Dimethylformamide (DMF) in >99.8% purity was supplied by Carl ROTH. HA in the form of cosmetic-grade powder was purchased from Aroma Zone France. Dispersants BYK 155/35 (a sodium polyacrylate-based dispersing additive for pigment stabilization in aqueous emulsion) and BYK ANTI-TERRA-250 (a controlled flocculating wetting and dispersing additive for aqueous primers) were provided by BYK. Zinc oxide (ZnO) in the form of powder <5 μ m and type A gelatin (GE), derived from pigskin, were supplied by Sigma-Aldrich. All chemicals were used as received.

2.2 | Formulation and Electrospinning

Before the electrospinning on the hydrogel film, the nanofibers were optimized. For this purpose, PLA was dissolved at, 10 wt% and 12.5 wt%, in acetone (AC) or in a mixture of two co-solvents, AC and DMF, at a ratio of 60:40 (wt/wt). Parameters of the process, such as voltage (40 kV and 60 kV) and distance from tip to collector (15 cm and 20 cm), were also investigated. The nanofibers were collected on aluminum foil, and the relative humidity was maintained at around 40%. For the production of nanofibers, a needleless electrospinning equipment such as Elmarco needleless machine known as 'NanoSpide' (model NS 1WS500U, Czech Republic) was used. This is an automated semi-industrial electrospinning machine capable of producing a continuous sample of nanofibers at a production rate of approximately 100 g/h per meter. The roll-to-roll system allowing the equipment to produce in continuity was not used, and the collector (aluminum sheet with or without the hydrogel) was fixed between two PMMA plates (the bottom plate being fenestrated). As far as experimental parameters were concerned, voltage and distance were variable, and the other parameters were fixed: carriage speed and flow rate. Each membrane was electrospun for 10 min.

After the optimization of fibers in terms of diameter and morphology, an amount corresponding to 5 wt% of IBU relative to

PLA was dissolved and into the PLA solution by mixing for 10 min under magnetic stirring.

2.3 | Elaboration of the Wound Dressing

The formation of a bilayer dressing involves a two-step procedure: first, a hydrogel layer was prepared; and then nanofibers were immediately electrospun onto it to construct a second layer.

- Step 1: To prepare the hydrogel film, 4 mg of BYK 155/35 or BYK ANTI-TERRA-250 (1.1 wt% compared to ZnO) were dissolved in 80 mL of distilled water. 0.36 g of ZnO particles were added to the solution and then mixed using an ultra-turrax for 10 min at 8000 rpm to disperse ZnO particles. Next, 8 g of GE were added to make a 10 wt% GE solution. In another flask, 0.5 g of HA was incorporated into 19.5 mL of distilled water to make a 2.5 wt% solution. The two solutions were stirred at 50°C to facilitate homogenization. 10 g of HA solution was then added to 17 g of GE solution and mixed. The solution was left in an airtight bottle overnight in an oven at 50°C to eliminate any bubbles. The liquid solution was then poured onto a sheet of Teflon and cast with a 300 μ m barcoater to form a film. Finally, the film was dried at room temperature (20°C) for 24 h.
- Step 2: The optimized solution of PLA was electrospun onto the hydrogel film with the accurate parameters. Before electrospinning, hydrogel films (10 $\times$ 5 cm) were placed on an aluminum foil acting as a collector surface and were moistened with distilled water; the excess of it was removed with Joseph paper. Nanofibers were deposited on the hydrogel substrate over a 10 min period, and then the complex was dried at room temperature in a closed fume hood overnight. Then, the bilayer dressing was detached from the aluminum and stored in normal temperature conditions.

Using this protocol, two kinds of wound dressing were produced, AL1 (Hydrogel/PLA), which designates the hydrogel without ZnO, and AL2 (Hydrogel/ZnO/PLA) containing ZnO particles.

2.4 | Characterization Techniques

2.4.1 | Light Scattering

A Malvern Mastersizer 3000 was used to determine the particle size of zinc oxide in water and to optimize the dispersant content (BYK 155/35 or BYK ANTI-TERRA-250) required to prevent the formation of agglomerates and improve the dispersion of zinc oxide particles in the GE solution.

2.4.2 | Scanning Electron Microscopy (SEM)

All the nanofiber samples and the bilayer structures were analyzed by SEM using a JEOL SEM (JSM-IT100) apparatus to

determine their morphological properties such as shape, homogeneity, and mean diameter. Samples were coated with a thin layer of gold by sputtering using a metal coating machine (POLARON E5100—France) to make them conductive. SEM observations were carried out for each sample with x500 and x5000 zoom. These images were then analyzed using ImageJ software to obtain the mean diameter of the nanofibers as well as their standard deviation, the percentage coefficient of variation, the diameter distribution, and the morphology of the fibers [25, 26].

2.4.3 | Image Analyzing

SEM observation of electrospun membrane were analyzed with ImageJ software and DiameterJ Plugin. All images were analyzed with a magnification of 500. The analysis is carried out on the entire image after black and white segmentation. All of the fibers are segmented in white, meaning the beads are an integral part of the fiber and the diameter of the fibers is a mean considering the bead.

2.4.4 | Differential Scanning Calorimetry (DSC)

The TA Q200 differential scanning calorimeter (DSC) was used to determine the thermal properties of PLA nanofibers. The samples were sealed in a non-hermetic aluminum pan. The experiments were carried out using 3 heating ramps, with initial heating from 20°C to 220°C, followed by cooling from 220°C to 20°C and heating from 20°C to 220°C at a rate of 10°C/min. Glass transition temperature was determined during the second heating ramp.

2.4.5 | Thermogravimetric Analysis (TGA)

In order to conclude on the incorporation of IBU but also on the evaporation of solvents, TGAs of pure IBU and PLA were done as references. Then, TGAs of PLA nanofibers with and without IBU were carried out for each kind of solvent. These analyses were performed using a TA Q500 apparatus under nitrogen at a heating rate of 10°C/min from 20°C to 800°C.

2.4.6 | Antimicrobial Activity

The antibacterial activity of the films AL1 (Hydrogel/PLA) and AL2 (Hydrogel/ZnO/PLA) for wound healing was assessed by time-kill assay on two bacterial strains: *Staphylococcus aureus* ATCC 25923 (Gram-positive) and *Escherichia coli* ATCC 25922 (Gram-negative). The method described by Pelin et al. [27] was adapted as follows: from fresh cultures obtained in Mueller Hinton broth, after a 24-h incubation at 37°C, microbial suspensions with a density of 0.5 McFarland (1.5×10^8 CFU—colony forming unit) were prepared in sterile saline solution. Each sample (cut 1 cm/1 cm and weighed) was placed in 2 mL of 0.5 McFarland microbial suspension. After 6, 24, 48, and 72 h of incubation at 37°C, 200 μ L of each combination was distributed into sterile Petri dishes, over which melted Mueller Hinton agar cooled to 45°C was added

and after homogenization and agar solidification, the plates were incubated for 24 h at 37°C and, when possible, the number of colonies was determined. To report the results per mL, the colony count is multiplied by five. In this study, AL1 was used as a negative control to be able to assess the improved antimicrobial activity of AL2. The interpretation of the results is based on the ability of substances with antibacterial activity to inhibit and/or kill bacterial cells. Following the contact between the microorganisms and the samples to be tested, the side effect translates into a decrease in the number of bacteria initially present in the 0.5 McFarland suspension, respectively in the number of colonies (each colony develops by multiplying a bacterial cell).

3 | Results and Discussion

3.1 | Parameters Influencing the Material Properties

3.1.1 | Optimization of Hydrogel Film

Hydrogel film was prepared as described previously. For the hydrogel film containing ZnO particles, as this latter is well known for its wound-healing properties, notably its antimicrobial and anti-inflammatory properties, and its ability to promote tissue regeneration [20, 21], it is important to ensure a homogeneous dispersion of the particles by adding a suitable dispersant. This dispersant will prevent the formation of agglomerates, optimizing the effectiveness of the dressing. Indeed, the deagglomeration of zinc oxide particles is essential to increase their specific surface area and therefore their antibacterial properties. In addition, their distribution within the hydrogel will be more homogeneous. ZnO content was fixed at 3 wt%, a concentration selected to ensure effective antimicrobial activity while maintaining the biocompatibility and structural integrity of the material [28, 29].

Two dispersants were selected, BYK 155–35 and BYK ANTI-TERRA-250, and compared at increasing weight content (0 wt% (reference), 0.5 wt%, 1 wt%, 1.5 wt%, and 2 wt% relative to the particle's weight). The analysis of the particle size in water (Figure 1) shows that the reference curve has a large distribution with a mean particle size of 2.13 μm , corresponding

to agglomerates of ZnO. Using a dispersant, the particle size of ZnO is smaller than the reference and, interestingly, BYK 155–35 tends to produce smaller ZnO particle sizes than ANTI-TERRA-250, with a wider distribution. Therefore, the dispersant BYK 155–35 at 1 wt% will be retained for the rest of the study due to its superior efficiency.

3.1.2 | Optimization of Electrospun Nanofibers

Numerous studies have investigated the electrospinning of PLA [26, 30–32], offering valuable insights into the nanofiber morphology and the key influencing parameters. The concentration of PLA, as well as the choice of solvents, directly affects the structure of the fibers. Depending on the solvent used, the fibers may not form at all, or may appear as beaded or unbeaded fibers, smooth or porous, with external and/or internal porosity. Generally, smooth fibers, with or without beads, and free of porosity, are interesting from a practical point of view. Indeed, the presence or absence of beads on the nanofibers can be an asset during the drug release. Acetone (AC) and Dimethylformamide (DMF) appear to be suitable, as these solvents, used alone or in combination, have been used to achieve the desired fiber morphologies [26, 31, 33]. Consequently, two concentrations of PLA; namely, 10 wt% and 12.5 wt%, were investigated with the use of pure acetone (AC) and a mixture of co-solvents AC:DMF (60:40 wt/wt). Concerning the process parameters, voltages of 40 kV and 60 kV, as well as distances of 15 cm and 20 cm between the wire and the collector, were chosen on the basis of the literature and experiments carried out previously by our research team [3, 27].

For all the solutions, the polymer was dissolved in the solvent at room temperature and stirred for 12 h before electrospinning. The carriage, where we put the solution, has a volume of 50 mL and its speed was 150 mm/s, and the wire 1 mm/s. The first nanofibers were obtained using a voltage of 40 kV and a distance of 15 cm; only the nature of solvents and concentration of PLA have been modified. From SEM images (Figure 2), the average diameters of the electrospun nanofibers were determined (Table 1) by image analysis with ImageJ software and DiameterJ Plugin. Consistent with the literature [33], it can be seen that PLA nanofibers electrospun onto aluminum foil from a 10 wt% concentration solution (Figure 2A,B) have a bead structure,

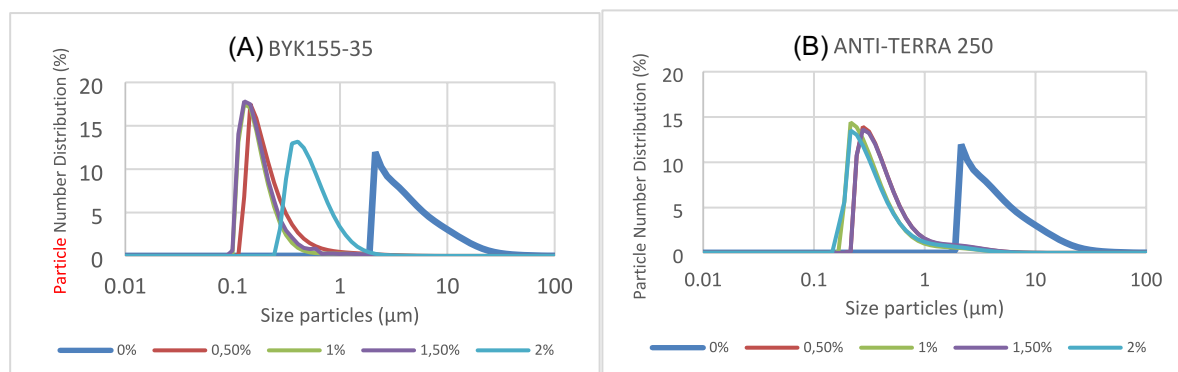


FIGURE 1 | Particle size distribution in presence of different dispersant contents determined by light scattering (A) BYK 155–35 (B) ANTI-TERRA-250. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.57726)]

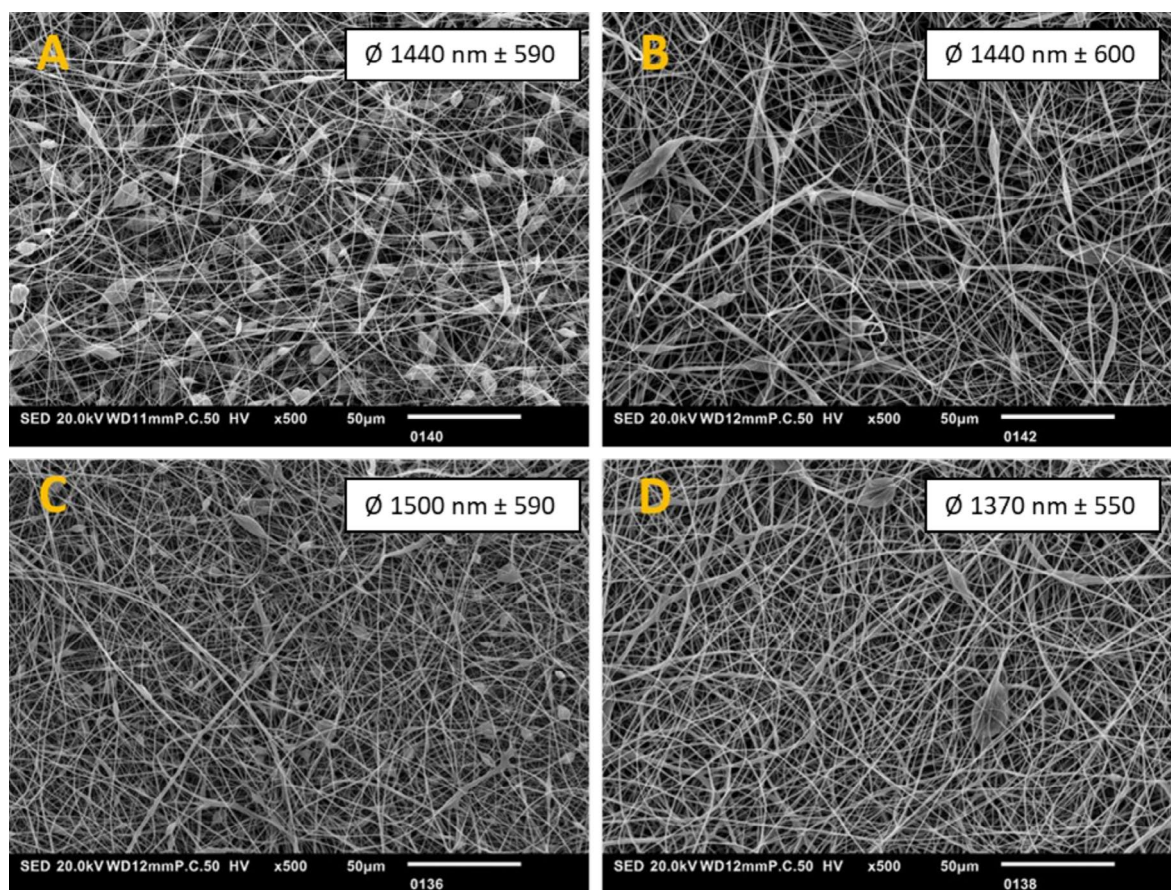


FIGURE 2 | SEM observation of PLA nanofiber, voltage: 40kV, distance 15cm (A) AC10wt% (B) AC12.5wt% (C) AC:DMF 10wt% (D) AC:DMF 12.5wt%. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

TABLE 1 | Mean diameter of the filament, electrospun at 40kV and 15cm with different solvents.

	AC		AC/DMF	
	10wt%	12,5wt%	10wt%	12,5wt%
Diameter (µm)	1.44	1.44	1.50	1.37
Standard deviation	0.59	0.60	0.59	0.55

whereas at 12.5wt% (Figure 2B,D), no beads were visible. However, the solvent does not have a significant impact on the morphology and the structure of the electrospun nanofibers. It was decided to keep both concentrations, 10wt% and 12.5wt% of PLA, in order to study the influence of the presence of beads in the release of active ingredients.

For a voltage of 60kV, the distances studied have no significant effect on the morphology of the nanofibers; beads can still be observed, but also a slight increase or decrease in the diameter of the nanofibers, as shown in Figures 3 and 4 and in Table 2. On the other hand, at 40kV, the distance has an impact on the morphology and on the diameter of the fiber. The amount of beads was more important at 15cm than at 20cm (PLA concentration = 10wt%), and the mean diameter of the fibers was about 1400nm at a distance of 15cm, while it was 700nm at 20cm.

It is consistent with the literature because a variation in distance will have an influence similar to a variation in tension. An increase in distance will generally reduce the diameter of the fiber, since the fiber's stretch is greater. On the other hand, if the distance increases beyond a certain critical threshold, the fiber diameter may increase again; this was not the case, due to the weaker electric field.

By comparing Figures 3 and 4, it can also be concluded that the voltage has no significant impact when AC was used; indeed, nanofibers retain a similar morphology with a 12.5% PLA/AC concentration. On the other hand, when AC:DMF was used, nanofibers appear to be strongly affected. At 60kV, the DMF-based solution produced fibers with a distinctive morphology, characterized by a very dense, interlaced network, suggesting incomplete evaporation of the solvent during electrospinning (Figure 5). In addition, no consistent values could be determined for these SEM images using image J software, because of the inability of the software to find a clear diameter on the treated image. The distribution of the nanofiber diameter was the best for a voltage of 40kV and a distance of 15cm, and of no matter the concentration of the solution.

Considering the results obtained, the rest of the study has been carried out with a voltage of 40kV and a distance of 15cm between the collector and the electrode wire. Due to the toxicity of DMF, acetone was chosen for the elaboration of the wound dressing. Further investigations focused on the effects

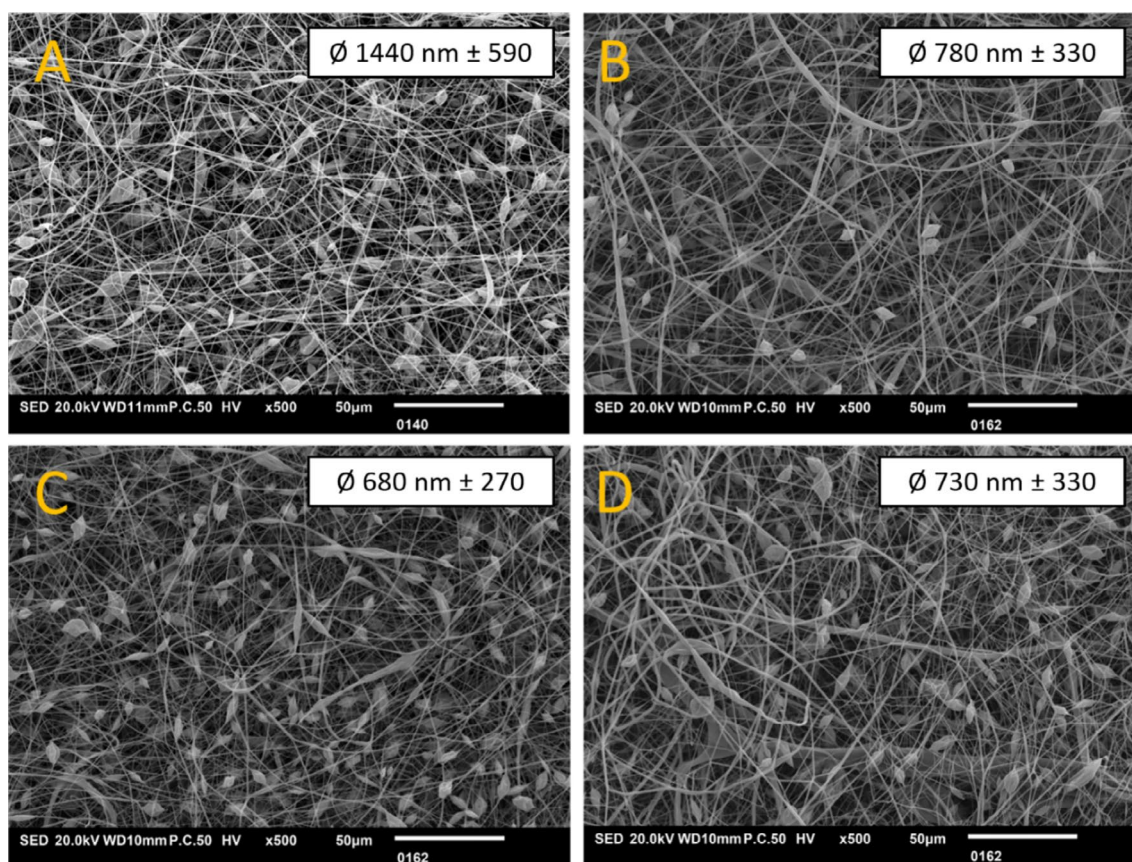


FIGURE 3 | SEM observation of PLA nanofiber, concentration 10wt%, solvent AC (A) 40kV—15cm (B) 40kV—20cm (C) 60kV—15cm (D) 60kV—20cm. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.57726)]

of electrospinning on wet hydrogel have been conducted, as well as the fibers produced following the incorporation of 5% IBU in PLA/AC solution.

3.2 | Elaboration of the Bilayer Wound Dressing

Solutions of PLA dissolved in acetone with and without IBU were electrospun on hydrogel film to evaluate the impact on the morphology of the electrospun membrane. The hydrogel is rehydrated prior to assembly in order to enhance interfacial interactions and improve adhesion between the hydrogel and the nanofiber scaffold.

Ibuprofen is a hydrophobic drug used to treat pain and inflammation in wounds. In order to develop electrospun scaffolds equipped with a drug delivery system for wound-healing applications, pure IBU was incorporated into PLA10wt% and PLA12.5wt% solutions. The concentration of IBU was set at 5wt% with respect to the PLA [3].

Figure 6 shows the SEM images of PLA nanofibers deposited on the hydrogel film. In comparison to the previously produced nanofiber membrane, electrospinning on a wet hydrogel induced notable changes, highlighting the strong influence of humidity on fiber porosity and morphology [34]. There were fewer beads in the membrane deposited on hydrogel, compared to Figures 3A and 6A.

Figure 6 shows that electrospinning solutions containing 5% IBU and 12.5wt% PLA onto the wet hydrogel produce nanofibers with a larger diameter than those without IBU (1100 nm against 760 nm) (Figure 6C,D). This increase is probably due to the change in viscosity of the solution but also due to the interactions between IBU and PLA. Some rheological studies should be done in the future. On the other hand, there is no significant difference with 10 wt% PLA (Figure 6A,B). Despite this increase in average fiber diameter, the fibers electrospun onto the hydrogel retained their smooth, fine, and continuous morphology. The PLA/IBU nanofibers are uniform in diameter; they have a dense and homogeneous arrangement, as well as sufficient length to create an interconnected network, making them ideal for applications such as dressings, filters, or other functional materials requiring a high specific surface area and good interconnection between fibers [35].

3.3 | Characterization of PLA Nanofibers

Thermal analysis of IBU-loaded nanofibers was carried out in order to confirm the presence of IBU in the PLA nanofibers. TGA analysis of pure IBU and pure PLA nanofibers was also done to provide a reference (Figure 7). The degradation curve of pure IBU showed that the degradation of the drug appeared at 205°C. In the degradation curves of 5% IBU/PLA, the presence of IBU was proved from the intermediate peaks noted at 204°C. Moreover, the quantification of IBU by TGA was found

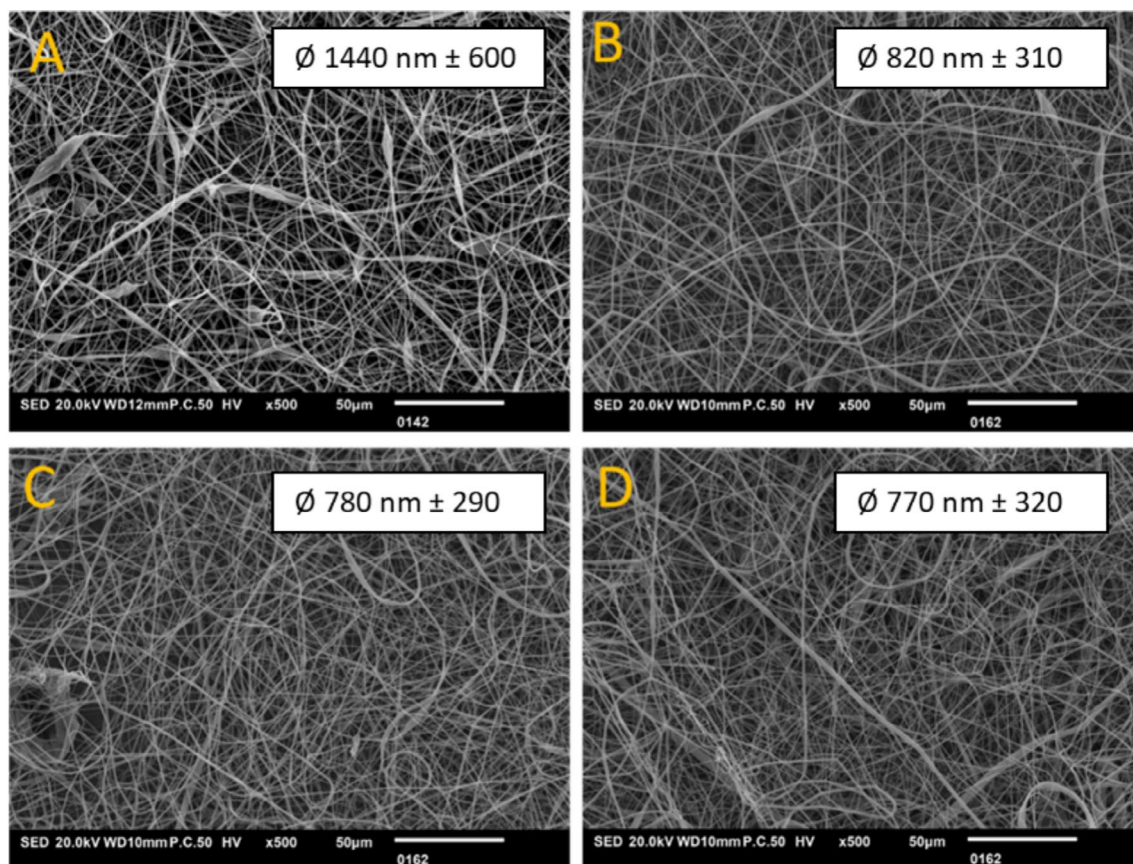


FIGURE 4 | SEM observation of PLA nanofiber, concentration 12.5 wt%, solvent AC (A) 40 kV–15 cm (B) 40 kV–20 cm (C) 60 kV–15 cm (D) 60 kV–20 cm. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.57726)]

TABLE 2 | Mean diameter of the filament, electrospun at 60 kV and AC with different distances.

	15 cm		20 cm	
	10 wt%	12,50 wt%	10 wt%	12,50 wt%
Diameter (µm)	0.68	0.78	0.73	0.77
Standard deviation	0.27	0.29	0.33	0.32

to be around 5%, in agreement with the amount of IBU loaded into the nanofibers. In addition, these TGAs highlight the absence of residual solvent in IBU-loaded PLA nanofibers.

DSC analyses show that PLA 12.5 wt% nanofibers have a glass transition temperature of 54°C, in line with literature data [36] indicating the transition of PLA from a glassy to a rubbery state (Figure 7). The absence of a visible melting peak at higher temperatures suggests that PLA is amorphous, in line with the fact that this polymer is rich in L-configuration lactic acid, which favors an amorphous structure. On cooling, no recrystallization is observed. This absence of major thermal transitions underlines the fact that PLA retains its amorphous structure after the heating and cooling cycle. Furthermore, the absence of an endothermic peak corresponding to solvent evaporation confirms the TGA results.

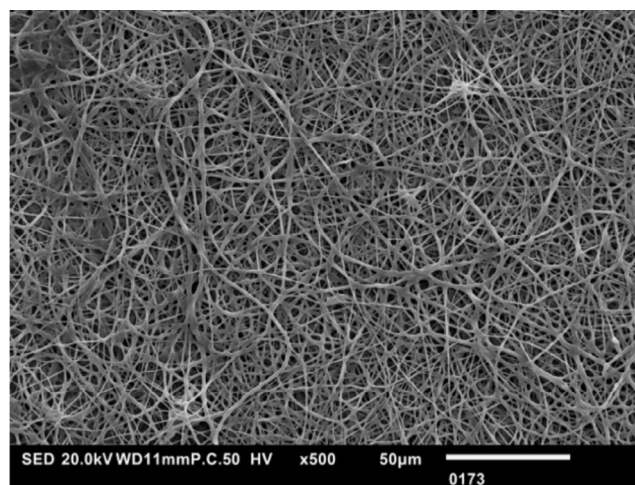


FIGURE 5 | SEM observation of PLA nanofibers, concentration 10 wt%, AC:DMF solvent, and 60 kV, 15 cm.

3.4 | Performance of the Material With Respect to Biomedical Application

3.4.1 | Antimicrobial Test

The electrospun wound dressings AL1 (Hydrogel/PLA10 wt%) and AL2 (Hydrogel/PLA10 wt%/ZnO) were tested on two bacterial strains (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922), both species being included in the category

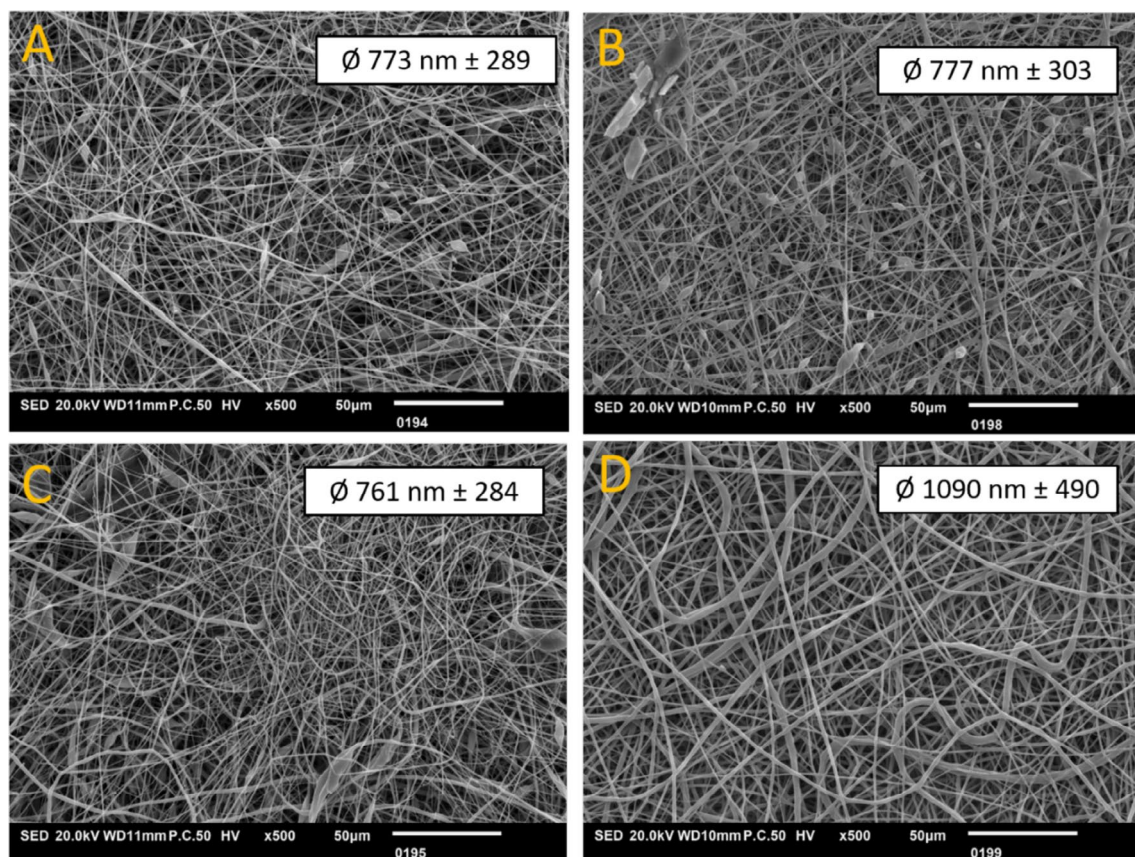


FIGURE 6 | SEM observation of PLA nanofibers deposited on hydrogel film (A) PLA10%; (B) PLA 10% and IBU; (C) PLA 12.5%; (D) PLA 12.5% and IBU. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

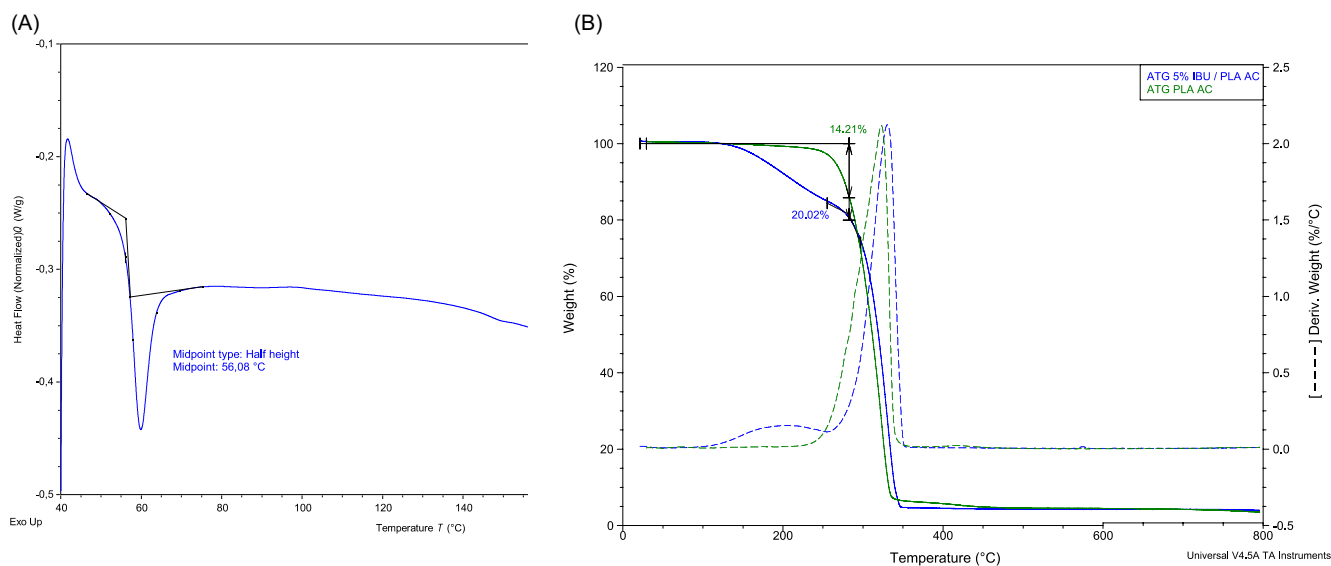


FIGURE 7 | (A) DSC curve of PLA 12wt% – first run (B) TGA curves of 5% IBU/PLA 12wt% (blue) and PLA 12wt%. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) opportunistic pathogenic microorganisms (Figures 8 and 9 and Table 3). Those species can be considered causative agents for many infectious processes with various locations, including the infection of surgical or other wounds. Also, their ability to produce biofilms and the presence of resistance

genes to different classes of substances used in anti-infective therapy further limit therapeutic alternatives.

Testing of AL1 on *S. aureus* shows antimicrobial activity only after 24h of contact (T2) with a logarithmic reduction of 99.998% at T4 (72h) (Figure 9). The constant decrease in the number of CFU/mL

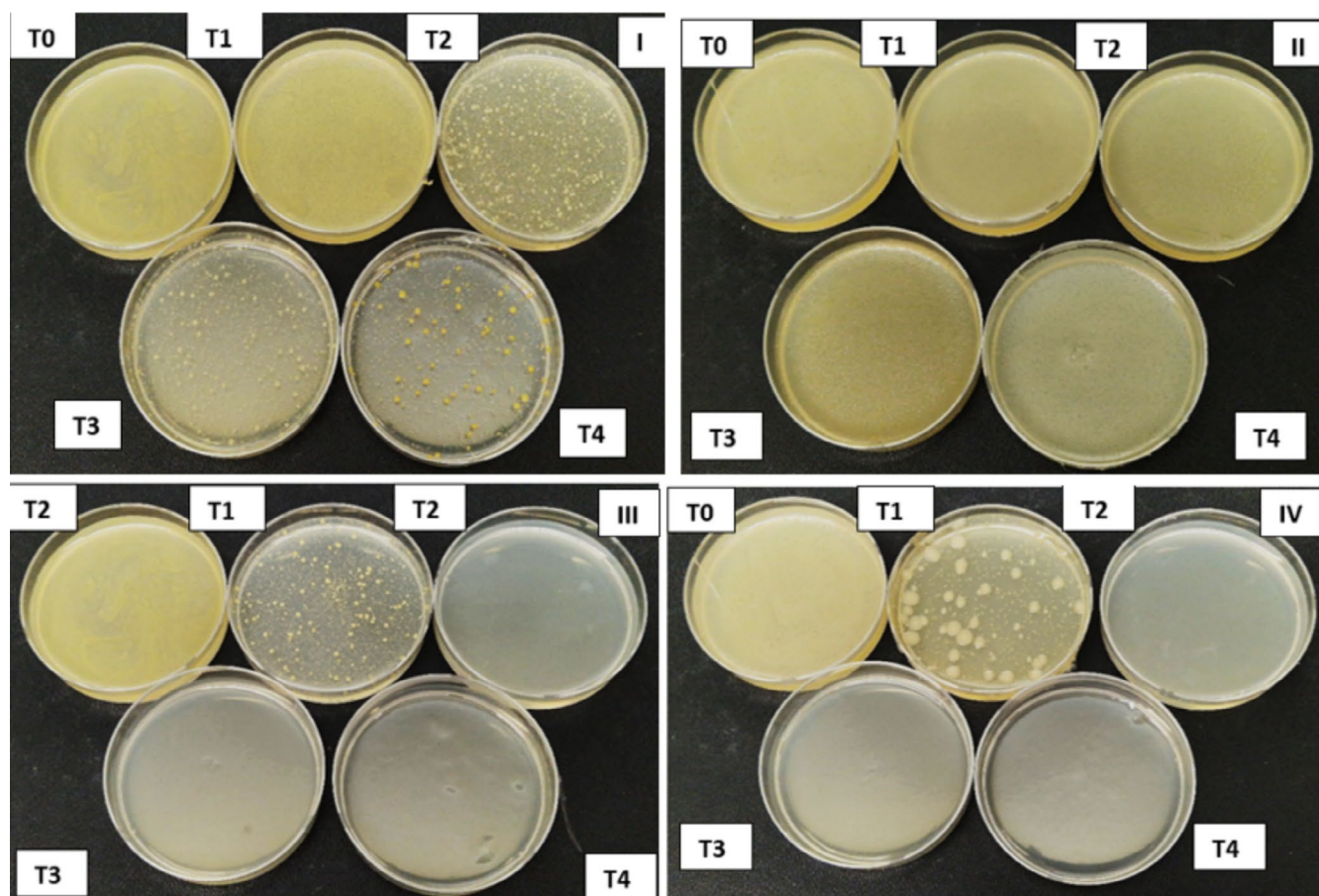


FIGURE 8 | Antimicrobial activity by time-kill assay: I. AL1 activity on *Staphylococcus aureus* ATCC 25923. II. AL1 activity on *Escherichia coli* ATCC 25922. III. AL2 activity on *Staphylococcus aureus* ATCC 25923. IV. AL2 activity on *Escherichia coli* ATCC 25922. T0 (initial number of bacterial cells), T1 (after 6 h), T2 (after 24 h), T3 (after 48 h), T4 (after 72 h). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

demonstrates the constant activity of the biologically active compound (Table 3). On the other hand, no activity could be demonstrated on the *E. coli* strain. HA is used for wound healing, and it was proved to inhibit microbes adhesion, including *Staphylococcus aureus* and biofilm formation, being considered with bacteriostatic dose-dependent effects [13]. Although the product containing only HA did not have an obvious antimicrobial effect on the *E. coli* strain, other studies have demonstrated its ability to inhibit the formation of biofilms if it has a looser structure that affects its mobility and development on surfaces [16].

AL2 product tested on *S. aureus* showed a much better antimicrobial activity, demonstrated from the T1 time (6 h) with a logarithmic reduction of 99.996%; and after 24 h, at the T2–T4 times no bacterial colony developed, which demonstrates a strong microbiocidal effect. The antimicrobial behavior on the *E. coli* strain is similar; after 24 h until 72 h, all microorganisms are non-viable. Various studies have demonstrated the antibacterial and antibiofilm effect of ZnO and ZnO particles [20–22] on both microbial strains taken in the study.

The study shows a very good activity of the bilayer dressing containing ZnO particles in the hydrogel layer compared to the dressing without ZnO particles. Although the antimicrobial effect against *S. aureus* is observed in both samples, the bilayer dressing without ZnO particles in the hydrogel layer shows a constant CFU reduction effect, but does not show a bactericidal

effect even after 72 h (Figure 8). The bilayer dressing containing ZnO particles in the hydrogel layer, on the other hand, demonstrates a logarithmic reduction of 100% at 24 h, an effect maintained at 72 h. As has been demonstrated in other studies, Gram-positive bacteria are more susceptible to the action of substances with antimicrobial action [27] *E. coli*, on the other hand, does not show any sensitivity to the bilayer dressing without ZnO particles in the hydrogel layer. The effect changes radically in the case of the bilayer dressing containing ZnO particles in the hydrogel layer, in which the effect is similar to that of *S. aureus*, with a logarithmic reduction of 100% from 24 to 72 h. In conclusion, adding ZnO to the bilayer dressing improved the antimicrobial effect against both bacterial strains.

4 | Conclusions

A new bilayer dressing was designed to offer controlled drug release, using a needleless electrospinning technique to deposit PLA nanofibers containing 5 wt% of IBU on a wet hydrogel film. The hydrogel film was composed of GE and HA, and ZnO particles were also incorporated. 1 wt% of BYK 155–35 was selected as the best dispersant content in order to avoid the agglomeration of ZnO particles in the aqueous GE solution. The use of acetone as a solvent allows the production of optimized PLA nanofibers, even at different distances (tip to collector) and voltages. Indeed, nanofibers produced from a solution of PLA/AC/IBU (5% wt/t)

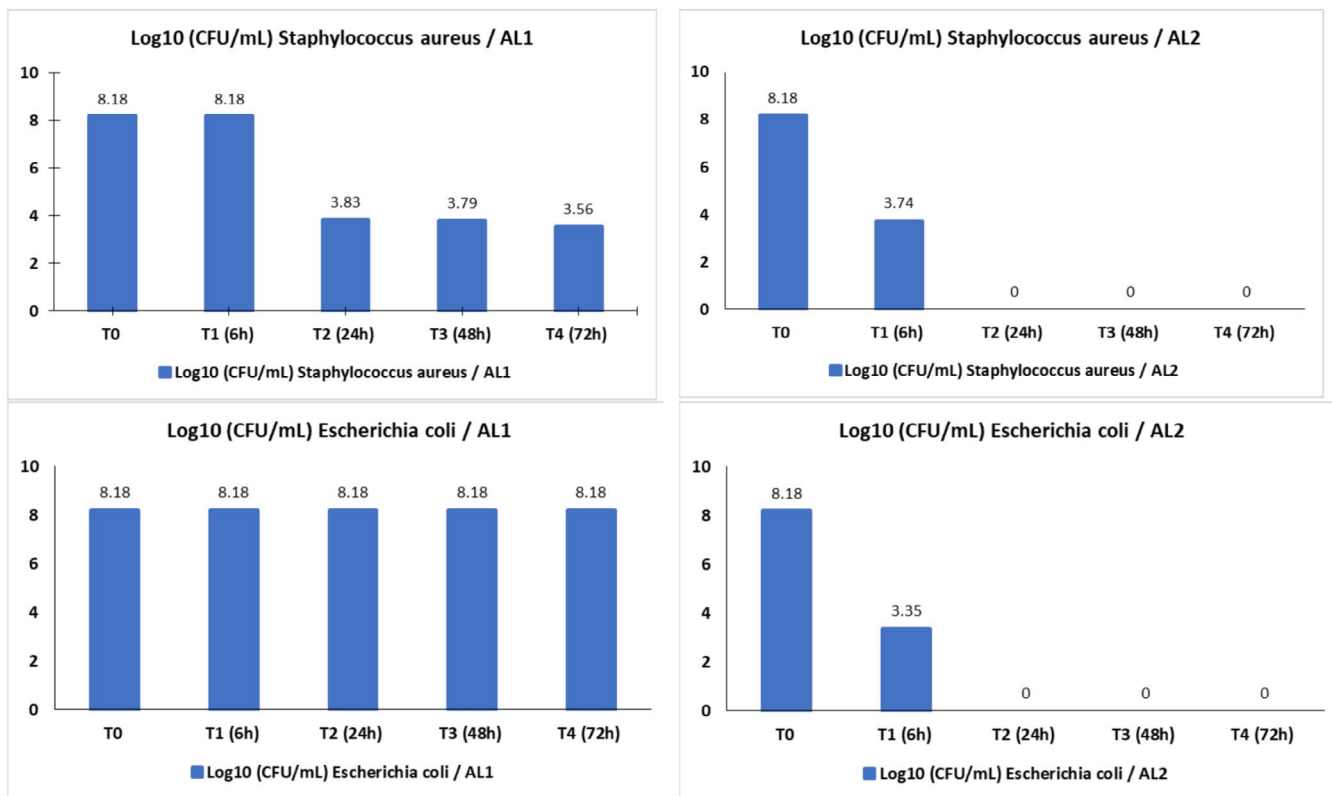


FIGURE 9 | Logarithmic reduction of *Staphylococcus aureus* and *Escherichia coli* populations after treatment with AL1 (Hydrogel/PLA) and AL2 (Hydrogel/PLA/ZnO). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.57726)]

TABLE 3 | Reduction of *Staphylococcus aureus* and *Escherichia coli* populations after treatment with AL1 (Hydrogel/PLA) and AL2 (Hydrogel/PLA/ZnO).

Time (h)	Sample	Bacteria species			
		<i>Staphylococcus aureus</i> ATCC 25293		<i>Escherichia coli</i> ATCC 25922	
		Log10 CFU/mL	% Log reduction	Log10 CFU/mL	% Log reduction
T0	AL1	8.18	0	8.18	0
	AL2	8.18	0	8.18	0
T1 (6h)	AL1	8.18	0	8.18	0
	AL2	3.74	99.996	3.35	99.998
T2 (24h)	AL1	3.83	99.995	8.18	0
	AL2	0	100	0	100
T3 (48h)	AL1	3.79	99.996	8.18	0
	AL2	0	100	0	100
T4 (72h)	AL1	3.56	99.998	8.18	0
	AL2	0	100	0	100

with the following parameters, voltage of 40kV and a distance of 15 cm onto wet hydrogel show a round, smooth, and homogeneous morphology, with an average diameter between 380 and 470 nm. Nanofibers with a PLA concentration of 10 wt% have beads, while beads were observed on those obtained with a PLA concentration of 12.5 wt%. TGA confirmed total evaporation of the solvent and the presence of IBU in the nanofibers. Finally,

an in vitro antimicrobial contact time analysis was conducted to evaluate the inhibition and/or eradication of bacterial cells over time in the presence of the bilayer dressing in cultures of *Staphylococcus aureus* and *Escherichia coli*. These results suggest that this dressing could be a promising candidate for a controlled drug delivery system for the treatment of inflammatory wounds. In addition, it can also reduce the frequency of dressing

changes, thereby improving patient adherence to the treatment. Future studies should explore the influence of nanostructure morphology on the controlled release profile of ibuprofen.

Author Contributions

Anne-Sophie Schuller: conceptualization (lead), funding acquisition (equal), project administration (equal), supervision (equal), validation (equal), visualization (equal), writing – original draft (lead), writing – review and editing (lead). **Élise Girault:** conceptualization (equal), resources (equal), supervision (equal), validation (equal), writing – original draft (equal). **Amdouni Sonia:** investigation (lead). **Leonard Ionut Atanase:** investigation (supporting), writing – original draft (supporting). **Cristina Elena Horhoge:** investigation (supporting), writing – original draft (supporting). **Floriane Leclinche:** conceptualization (supporting), writing – original draft (supporting). **Christelle Delaite:** conceptualization (supporting), funding acquisition (lead), supervision (equal), validation (equal), writing – original draft (supporting).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request.

References

1. S. Wu, T. Dong, Y. Li, et al., “State-Of-The-Art Review of Advanced Electrospun Nanofiber Yarn-Based Textiles for Biomedical Applications,” *Applied Materials Today* 27 (2022): 101473, <https://doi.org/10.1016/j.apmt.2022.101473>.
2. C.-H. Yao, C.-Y. Lee, C.-H. Huang, Y.-S. Chen, and K.-Y. Chen, “Novel Bilayer Wound Dressing Based on Electrospun Gelatin/Keratin Nanofibrous Mats for Skin Wound Repair,” *Materials Science & Engineering, C, Materials for Biological Applications* 79 (2017): 533–540, <https://doi.org/10.1016/j.msec.2017.05.076>.
3. T. Riaz, N. Gull, A. Islam, M. Dilshad, L. Atanase, and C. Delaite, “Needleless Electrospinning of Poly (ε-Caprolactone) Nanofibers Deposited on Gelatin Film for Controlled Release of Ibuprofen,” *Chemical Papers* 77 (2023): janv–2669, <https://doi.org/10.1007/s11696-022-02655-6>.
4. M. Gizaw, J. Thompson, A. Faglie, S.-Y. Lee, P. Neuenschwander, and S.-F. Chou, “Electrospun Fibers as a Dressing Material for Drug and Biological Agent Delivery in Wound Healing Applications,” *Bioengineering* 5, no. 1 (2018): 9, <https://doi.org/10.3390/bioengineering5010009>.
5. E.-R. Kenawy, G. L. Bowlin, K. Mansfield, et al., “Release of Tetracycline Hydrochloride From Electrospun Poly(Ethylene-Co-Vinylacetate), poly(Lactic Acid), and a Blend,” *Journal of Controlled Release* 81, no. 1–2 (2002): 57–64, [https://doi.org/10.1016/s0168-3659\(02\)00041-x](https://doi.org/10.1016/s0168-3659(02)00041-x).
6. R. Khan, M. U. Aslam Khan, G. M. Stojanović, A. Javed, S. Haider, and S. I. Abd Razak, “Fabrication of Bilayer Nanofibrous-Hydrogel Scaffold From Bacterial Cellulose, PVA, and Gelatin as Advanced Dressing for Wound Healing and Soft Tissue Engineering,” *ACS Omega* 9, no. 6 (2024): 6527–6536, <https://doi.org/10.1021/acsomega.3c06613>.
7. A. Nazir, N. Khenoussi, L. Schacher, T. Hussain, D. Adolphe, and A. H. Hekmati, “Using the Taguchi Method to Investigate the Effect of Different Parameters on Mean Diameter and Variation in PA-6 Nanofibres Produced by Needleless Electrospinning,” *RSC Advances* 5, no. 94 (2015): 76892–76897, <https://doi.org/10.1039/C5RA13649K>.
8. S. M. Tan, X. Y. Teoh, J. le Hwang, et al., “Electrospinning and Its Potential in Fabricating Pharmaceutical Dosage Form,” *Journal of Drug Delivery Science and Technology* 76 (2022): 103761, <https://doi.org/10.1016/j.jddst.2022.103761>.
9. D. Li, W. Chen, B. Sun, et al., “A Comparison of Nanoscale and Multiscale PCL/Gelatin Scaffolds Prepared by Disc-Electrospinning,” *Colloids and Surfaces B: Biointerfaces* 146 (2016): 632–641, <https://doi.org/10.1016/j.colsurfb.2016.07.009>.
10. L. Vysloužilová, M. Buzgo, P. Pokorný, et al., “Needleless Coaxial Electrospinning: A Novel Approach to Mass Production of Coaxial Nanofibers,” *International Journal of Pharmaceutics* 516, no. 1 (2017): 293–300, <https://doi.org/10.1016/j.ijpharm.2016.11.034>.
11. M. Kouhi, M. Mobasheri, and A. Valipouri, “Chapter 7 – Needleless Electrospinning,” in *Electrospun and Nanofibrous Membranes*, ed. A. Kargari, T. Matsuura, and M. M. A. Shirazi (Elsevier, 2023), 145–171, <https://doi.org/10.1016/B978-0-12-823032-9.00011-8>.
12. J. Liu, T. Li, H. Zhang, et al., “Electrospun Strong, Bioactive, and Bioabsorbable Silk Fibroin/Poly (L-Lactic-Acid) Nanoyarns for Constructing Advanced Nanotextile Tissue Scaffolds,” *Materials Today Bio* 14 (2022): 100243, <https://doi.org/10.1016/j.mtbio.2022.100243>.
13. F. Zamboni, C. Okoroafor, M. P. Ryan, et al., “On the Bacteriostatic Activity of Hyaluronic Acid Composite Films,” *Carbohydrate Polymers* 260 (2021): 117803, <https://doi.org/10.1016/j.carbpol.2021.117803>.
14. J. Li, A. He, C. C. Han, D. Fang, B. S. Hsiao, and B. Chu, “Electrospinning of Hyaluronic Acid (HA) and HA/Gelatin Blends,” *Macromolecular Rapid Communications* 27, no. 2 (2006): 114–120, <https://doi.org/10.1002/marc.200500726>.
15. J. B. Leach, K. A. Bivens, C. W. Patrick, Jr., and C. E. Schmidt, “Photocrosslinked Hyaluronic Acid Hydrogels: Natural, Biodegradable Tissue Engineering Scaffolds,” *Biotechnology and Bioengineering* 82, no. 5 (2003): 578–589, <https://doi.org/10.1002/bit.10605>.
16. A. Vigué, D. Vautier, A. Kaytoue, et al., “*Escherichia coli* Biofilm Formation, Motion and Protein Patterns on Hyaluronic Acid and Polydimethylsiloxane Depend on Surface Stiffness,” *Journal of Functional Biomaterials* 13, no. 4 (2022): 237, <https://doi.org/10.3390/jfb13040237>.
17. B. Ekram, “Review: Functionalization of Biopolymer-Based Electrospun Nanofibers for Wound Healing,” *Journal of Materials Science* 60, no. 20 (2025): 8308–8342, <https://doi.org/10.1007/s10853-025-10931-5>.
18. K. R. Raghupathi, R. T. Koodali, and A. C. Manna, “Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles,” *Langmuir* 27, no. 7 (2011): 4020–4028, <https://doi.org/10.1021/la104825u>.
19. N. Padmavathy and R. Vijayaraghavan, “Enhanced Bioactivity of ZnO Nanoparticles-An Antimicrobial Study,” *Science and Technology of Advanced Materials* 9, no. 3 (2008): 035004, <https://doi.org/10.1088/1468-6996/9/3/035004>.
20. A. Abdelghafar, N. Yousef, and M. Askoura, “Zinc Oxide Nanoparticles Reduce Biofilm Formation, Synergize Antibiotics Action and Attenuate *Staphylococcus aureus* Virulence in Host; an Important Message to Clinicians,” *BMC Microbiology* 22, no. 1 (2022): 244, <https://doi.org/10.1186/s12866-022-02658-z>.
21. U. Kadiyala, E. S. Turali-Emre, J. H. Bahng, N. A. Kotov, and J. S. VanEpps, “Unexpected Insights Into Antibacterial Activity of Zinc Oxide Nanoparticles Against Methicillin Resistant *Staphylococcus Aureus* (MRSA),” *Nanoscale* 10, no. 10 (2018): 4927–4939, <https://doi.org/10.1039/C7NR08499D>.
22. M. Rihacek, L. Kosaristanova, T. Fialova, et al., “Zinc Effects on Bacteria: Insights From *Escherichia coli* by Multi-Omics Approach,” *mSystems* 8, no. 6 (2023): e0073323, <https://doi.org/10.1128/msystems.00733-23>.
23. M. Khandaker, S. Riahinezhad, H. G. Jamadagni, T. L. Morris, A. V. Coles, and M. B. Vaughan, “Use of Polycaprolactone Electrospun

- Nanofibers as a Coating for Poly(Methyl Methacrylate) Bone Cement," *Nanomaterials* 7, no. 7 (2017): 175, <https://doi.org/10.3390/nano7070175>.
24. R. Abdulhussain, A. Adebisi, B. R. Conway, and K. Asare-Addo, "Electrospun Nanofibers: Exploring Process Parameters, Polymer Selection, and Recent Applications in Pharmaceuticals and Drug Delivery," *Journal of Drug Delivery Science and Technology* 90 (2023): 105156, <https://doi.org/10.1016/j.jddst.2023.105156>.
25. A. Greiner and J. H. Wendorff, "Electrospinning: A Fascinating Method for the Preparation of Ultrathin Fibers," *Angewandte Chemie International Edition* 46, no. 30 (2007): 5670–5703, <https://doi.org/10.1002/anie.200604646>.
26. A. Jahangir, T. Rumi, M. Wahab, E. Khan, M. Rahman, and Z. Sayed, "Poly Lactic Acid (PLA) Fibres: Different Solvent Systems and Their Effect on Fibre Morphology and Diameter," *American Journal of Chemistry* 2017 (2017): 177–186, <https://doi.org/10.5923/j.chemistry.20170706.01>.
27. I. M. Pelin, M. Sillion, I. Popescu, C. M. Rîmbu, G. Fundueanu, and M. Constantin, "Pullulan/Poly(Vinyl Alcohol) Hydrogels Loaded With *Calendula Officinalis* Extract: Design and in Vitro Evaluation for Wound Healing Applications," *Pharmaceutics* 15, no. 6 (2023): 1674, <https://doi.org/10.3390/pharmaceutics15061674>.
28. D. M. Rata, A. N. Cadinoiu, M. Popa, et al., "Biocomposite Hydrogels for the Treatment of Bacterial Infections: Physicochemical Characterization and in Vitro Assessment," *Pharmaceutics* 13, no. 12 (2021): 2079, <https://doi.org/10.3390/pharmaceutics13122079>.
29. D. M. Rata, A. N. Cadinoiu, O. M. Daraba, L. M. Gradinaru, L. I. Atanase, and D. L. Ichim, "Influence of ZnO Nanoparticles on the Properties of Ibuprofen-Loaded Alginate-Based Biocomposite Hydrogels With Potential Antimicrobial and Anti-Inflammatory Effects," *Pharmaceutics* 15, no. 9 (2023): 2240, <https://doi.org/10.3390/pharmaceutics15092240>.
30. Q. Zheng, Y. Xi, and Y. Weng, "Functional Electrospun Nanofibers: Fabrication, Properties, and Applications in Wound-Healing Process," *RSC Advances* 14, no. 5 (2024): 3359–3378, <https://doi.org/10.1039/D3RA07075A>.
31. R. Casasola, N. L. Thomas, A. Trybala, and S. Georgiadou, "Electrospun Poly Lactic Acid (PLA) Fibres: Effect of Different Solvent Systems on Fibre Morphology and Diameter," *Polymer* 55, no. 18 (2014): 4728–4737, <https://doi.org/10.1016/j.polymer.2014.06.032>.
32. S. Georgiadou, "Electrospun Poly Lactic Acid (PLA) Fibres: Effect of Different Solvent Systems on Fibre Morphology and Diameter," *Polymer* 2014 Consulté le: 5 mars 2025. [En ligne]. Disponible sur, https://www.academia.edu/101164401/Electrospun_poly_lactic_acid_PLA_fibres_Effect_of_different_solvent_systems_on_fibre_morphology_and_diameter.
33. C. Huang and N. Thomas, "Fabricating Porous Poly(Lactic Acid) Fibres via Electrospinning," *European Polymer Journal* 99 (2018): 464–476, <https://doi.org/10.1016/j.eurpolymj.2017.12.025>.
34. G. Yazgan, R. I. Dmitriev, V. Tyagi, et al., "Steering Surface Topographies of Electrospun Fibers: Understanding the Mechanisms," *Scientific Reports* 7, no. 1 (2017): 158, <https://doi.org/10.1038/s41598-017-00181-0>.
35. D. Reneker and A. Yarin, "Electrospinning Jets and Polymer Nanofibers," *Polymer* 49 (2008): 2387–2425, <https://doi.org/10.1016/j.polymer.2008.02.002>.
36. A. A. Marek and V. Verney, "Photochemical Reactivity of PLA at the Vicinity of Glass Transition Temperature. The Photo-Rheology Method," *European Polymer Journal* 81 (2016): 239–246, <https://doi.org/10.1016/j.eurpolymj.2016.06.016>.