

Production and characterization of a double-layered wound dressing containing electrospun polylactic acid and hypericum-infused alginate hydrogel

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This study presents the development of a double-layered, natural-based wound dressing designed to enhance healing and reduce infection risk. The aim is to combine key dressing properties—moisture retention, high absorption capacity, optimal porosity, and antimicrobial activity—within a synergistic structure. The outer layer was fabricated from polylactic acid (PLA) *via* electrospinning. Characterization results revealed a highly porous structure (>85%) and a contact angle of 128°, indicating hydrophobic behavior that provides an effective barrier against external fluids while maintaining gas permeability. Mechanical testing demonstrated a tensile strength of approximately 4 MPa, ensuring sufficient durability for practical applications. Morphological analyses using scanning electron microscopy (SEM) and optical microscopy confirmed uniform nanofiber distribution and structural integrity, while Fourier-transform infrared (FTIR) analysis verified the preservation of PLA's chemical structure after electrospinning. The inner layer consists of an alginate-based hydrogel, selected for its excellent moisture retention and biocompatibility. This layer was functionalized with Hypericum perforatum extract at concentrations ranging from 1% to 4%. Swelling studies showed that the 2% extract formulation exhibited the highest swelling capacity (652%), along with improved flexibility and reduced hardness. FTIR results confirmed the successful incorporation of the extract into the hydrogel matrix. Overall, the combination of mechanically robust PLA nanofibers and bioactive, highly absorbent hydrogel demonstrates a promising double-layered wound dressing with potential applications in advanced wound care and tissue engineering.

Keywords: Alginate hydrogel, Bilayer wound dressing, Electrospinning, *Hypericum perforatum* extract, Polylactic acid

INTRODUCTION

Skin is the largest organ of the human body and acts as the first protective barrier against external pathogens, mechanical injuries, and environmental stressors. Damage to skin tissue caused by burns, surgical interventions, trauma, or chronic diseases may disrupt this barrier and significantly increase the risk of infection and delayed healing. Therefore, the development of advanced wound dressing materials capable of accelerating tissue regeneration while preventing microbial contamination has attracted considerable attention in recent years [1, 2].

An ideal wound dressing should maintain a moist environment, absorb excess wound exudate, permit oxygen exchange, prevent bacterial invasion, and possess sufficient mechanical integrity [3]. Traditional wound dressings such as cotton gauze often fail to provide these multifunctional properties because of their low bioactivity and poor moisture management capacity [4]. Consequently, modern wound care approaches increasingly focus on bioactive and multifunctional dressing systems that can mimic the extracellular matrix (ECM) and support cellular activities during healing [5].

Electrospun nanofibrous membranes have emerged as promising materials for wound healing applications due to their high porosity, interconnected structure, and large surface-area-to-volume ratio [6]. These characteristics enable effective gas permeability, fluid management, and cell attachment while structurally resembling the native ECM [7]. Among biodegradable polymers, polylactic acid (PLA) has been extensively investigated because of its biodegradability, biocompatibility, non-toxicity, and favorable mechanical properties [8]. Electrospun PLA membranes additionally provide hydrophobic barrier properties that can protect wounds against external fluid penetration while maintaining adequate breathability [9].

Hydrogels are another widely studied class of wound dressing materials owing to their excellent swelling behavior, flexibility, and capacity to maintain a moist wound environment [10]. Sodium alginate, a naturally derived polysaccharide extracted from brown algae, is particularly attractive for biomedical applications due to its biocompatibility, non-toxicity, and mild gelation ability in the presence of divalent cations such as

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Ca²⁺ [11]. Alginate hydrogels can efficiently absorb wound exudates and promote tissue regeneration through their soft and hydrated structure [12].

Recently, increasing attention has been directed toward incorporating natural bioactive compounds into wound dressings to improve antibacterial and healing performance. *Hypericum perforatum* L. (St. John's Wort) is a medicinal plant known for its antimicrobial, antioxidant, anti-inflammatory, and tissue regenerative activities [13]. The therapeutic effects of *Hypericum* extract are mainly associated with bioactive compounds such as hypericin, hyperforin, and flavonoids [14]. Previous studies have demonstrated that *Hypericum*-based formulations can enhance fibroblast proliferation, collagen formation, and wound contraction while reducing microbial growth [15, 16].

Although electrospun nanofibers and hydrogel systems have individually demonstrated promising wound healing performance, studies focusing on bilayer structures that combine the protective advantages of electrospun PLA membranes with the bioactive and highly absorbent characteristics of *Hypericum*-loaded alginate hydrogels remain limited. Therefore, the present study aims to develop and characterize a double-layered wound dressing composed of an electrospun PLA outer layer and a *Hypericum* extract-incorporated alginate hydrogel inner layer. The structural, chemical, mechanical, swelling, wettability, and antibacterial properties of the developed system were comprehensively investigated to evaluate its potential for advanced wound care applications.

EXPERIMENTAL

Materials

Poly(lactic acid) (PLA 2003D, Mw: 120,000–160,000 g/mol) was procured from NatureWorks. Dimethylformamide (DMF) and chloroform (CHCl₃) were acquired from Isolab (Turkey). Methanol, ethanol, sodium alginate (alginic acid sodium salt, Sigma-Aldrich, CAS No: 9005-38-3), and poly(ethylene glycol) 400 (PEG 400) were purchased from Merck Chemicals (Turkey). St. John's Wort extract (*Hypericum perforatum* L.) was obtained from Selen Biokimya (Istanbul, Turkey) with lot number S007.R40101.G0034.231220.

Fabrication of electrospun PLA membranes

The outer protective layer of the bilayer wound dressing was produced using an electrospinning machine (IDASONIC NanoWeaver®-Pro). A polymer solution was prepared by dissolving 12.5 wt% PLA in a solvent system consisting of DMF and chloroform (70% chloroform by volume) and stirred

for 4 h at 50 °C to ensure complete dissolution. To improve the fiber morphology and act as a pore forming agent, 20 wt% PEG 400 (relative to the PLA weight) was incorporated into the homogeneous solution and stirred at room temperature [17]. The electrospinning process was conducted under optimized conditions to ensure bead-free and uniform nanofibers. The solution was fed at a constant flow rate of 0.02 mL/min through a syringe pump. A high voltage of 18 kV was applied, and the distance between the needle tip and the flat aluminum collector was maintained at 18 cm. The production was carried out for 15 min to reach the desired membrane thickness. Figure 1 shows a schematic diagram of the formation of the outer layer of the PLA electrospun fiber via the electrospinning process.

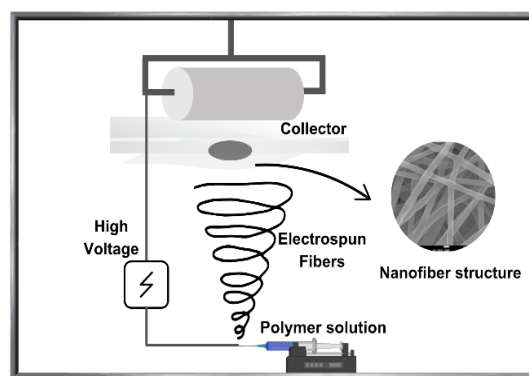


Figure 1. Schematic illustration of the electrospinning setup used for producing the protective PLA outer layer of the bilayer wound dressing.

Preparation of alginate hydrogels containing *Hypericum* extract

The inner healing layer, composed of sodium alginate (SA) hydrogel, was enriched with varying concentrations of St. John's Wort extract. A 1% (w/v) SA solution was initially prepared in distilled water at 50 °C. The St. John's Wort extract was dissolved in a 70:30 ethanol-water mixture and added to the SA solution at ratios ranging from 1% to 4% (v/v). The mixture was then homogenized using an ultrasonic homogenizer to ensure uniform distribution of the plant extract. The solution was cast into petri dishes and ionically cross-linked via the dropwise addition of 0.03 M CaCl₂. Following the gelation process, the hydrogels were washed with methanol to eliminate unreacted residues and rinsed three times with distilled water to remove excess Ca²⁺ ions and remaining solvents [18]. The visual appearances of the prepared hydrogels are shown in Figure 2, where A0 represents the pure alginate (control), and A1, A2, A3, and A4

correspond to the hydrogels enriched with 1%, 2%, 3%, and 4% *Hypericum* extract, respectively.

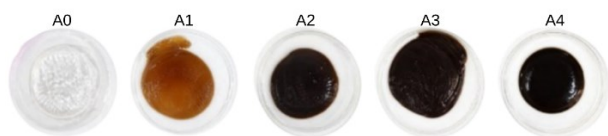


Figure 2. Physical appearance of the synthesized hydrogel samples: Pure alginate (A0) and *Hypericum* extract-incorporated alginate hydrogels (A1–A4).

RESULTS AND DISCUSSION

The surface morphology and structural characteristics of the electrospun membranes were evaluated using scanning electron microscopy (SEM) and optical microscopy. Figure 3 presents the SEM and optical microscope images of the PLA membrane at various magnification ratios. The SEM analysis reveals that the PLA membrane possesses high porosity, a homogeneous fiber distribution, and well-maintained structural integrity. Furthermore, the absence of significant beading indicates that the electrospinning parameters were optimally selected. High-magnification SEM images show that the fibers have a smooth and even surface, forming an interconnected, porous network structure. These properties contribute to the membrane's gas and water vapor permeability while providing a barrier behavior that restricts liquid passage. The optical microscope images corroborate the fibrous and porous architecture observed in the SEM analysis, confirming a homogeneous overall surface and structural continuity. The fibers are randomly but stably distributed, with no excessive concentration or dilution in specific regions, indicating a successful production process. The obtained bead-free and uniformly distributed PLA nanofibers are consistent with previous electrospinning studies reported in the literature. Similar smooth and interconnected PLA nanofibrous structures were observed by Abrigo *et al.* [7], who stated that optimized electrospinning parameters significantly improve fiber homogeneity and porosity, which are essential for wound healing applications.

The chemical structures and functional groups of the PLA membranes and bioactive hydrogels were identified using Fourier transform infrared spectroscopy (FTIR), as presented in Figure 4. For the alginate-based hydrogels (A0–A4), the broad absorption band between 3200–3500 cm^{-1} is attributed to the stretching vibrations of hydroxyl (OH^-) groups, indicating the polysaccharide nature and water content within the alginate chains.

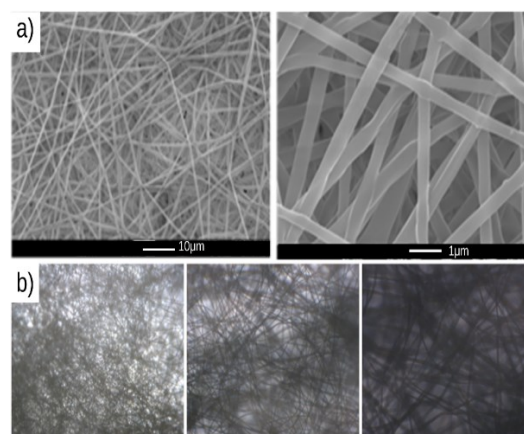


Figure 3. SEM (a) and optical microscopy (b) images of electrospun PLA membranes.

The characteristic peaks at 1600 cm^{-1} and 1000–1100 cm^{-1} correspond to the carboxylate groups ($-\text{COO}^-$) and the C–O–C stretching of the G and M blocks, respectively [19]. With increasing concentrations of *Hypericum* extract (A1–A4), a notable increase in peak intensity at 1400 cm^{-1} was observed, suggesting enhanced interactions between the phenolic components of the extract and the alginate carboxyl groups. The intensified OH^- band in the enriched samples further confirms the successful integration of the extract through hydrogen bonding [20]. Regarding the PLA membrane, the intense and sharp peak at 1750–1760 cm^{-1} represents the C=O stretching, serving as the primary evidence for the ester structure of PLA. The range of 1080–1185 cm^{-1} , known as the "fingerprint region," shows strong C–O and C–O–C asymmetric vibrations characteristic of PLA [8]. Peaks at 2995 cm^{-1} and 2945 cm^{-1} correspond to asymmetric and symmetric $-\text{CH}$ stretching, while the broad, weak peak at 3400–3600 cm^{-1} is attributed to the $-\text{OH}$ stretching of the PEG 400 chain ends. The results indicate that the high-voltage electrospinning process and solvent evaporation did not lead to chemical degradation or undesired side reactions.

The swelling tests of pure (A0) and *Hypericum* extract-incorporated (A1–A4) alginate hydrogels were evaluated, and the results are summarized in Figure 5. All samples exhibited a time-dependent increase in their swelling ratios, reaching equilibrium within 24 h. This behavior is primarily attributed to the high hydrophilicity of the alginate polymer chains, while the CaCl_2 cross-linking mechanism effectively regulated the overall water absorption capacity. The addition of *Hypericum* extract significantly altered the swelling performance.

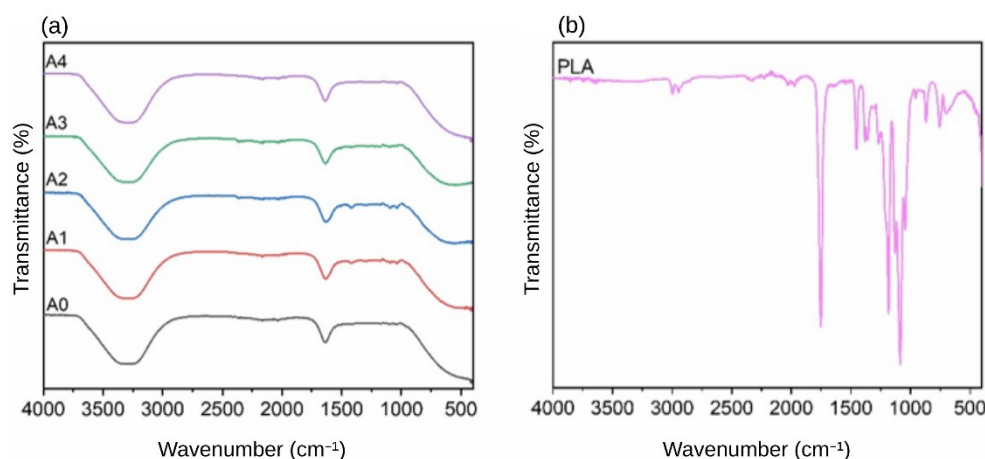


Figure 4. FTIR spectra of (a) *Hypericum* extract-incorporated alginate hydrogels and (b) electrospun PLA membranes.

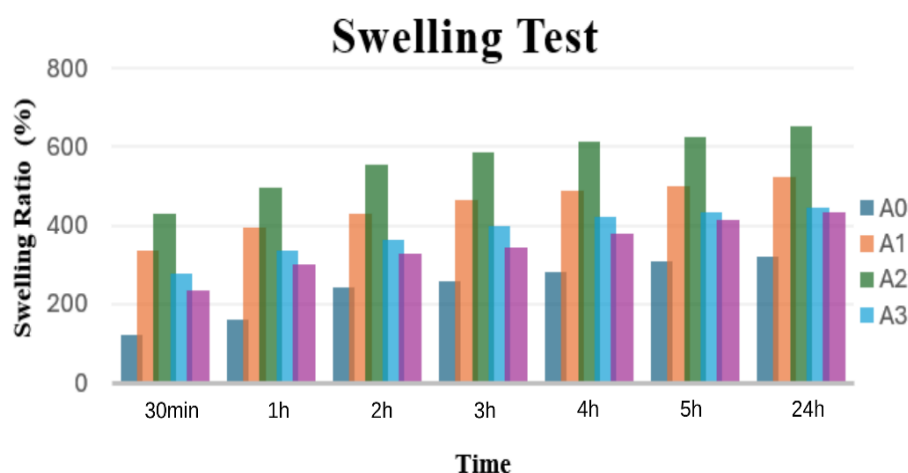


Figure 5. Percent swelling ratios of hydrogel samples at different time intervals

The A2 formulation (2% extract) achieved the highest swelling ratio of 652%. This enhancement is likely due to the phenolic and flavonoid components within the extract forming additional hydrogen bonds, which expand the hydrogel matrix and facilitate water uptake. However, a decrease in swelling was observed at higher concentrations (A3 and A4). This reduction stems from increased molecular interactions and enhanced Ca²⁺ binding activity, leading to a more densely packed and rigid network. This tighter structure restricts water diffusion into the matrix, thereby limiting the total swelling capacity. The high swelling behavior of alginate-based hydrogels is mainly associated with the hydrophilic hydroxyl and carboxyl groups present in the polymer backbone. Similar swelling trends were reported by Ahmed [10], who demonstrated that hydrogel swelling capacity strongly depends on crosslinking density and polymer-network flexibility. The enhanced swelling observed in the A2 formulation may be attributed to the phenolic compounds in *Hypericum* extract, which likely increased intermolecular spacing and water uptake capacity.

The structural properties of the electrospun PLA membranes were evaluated through swelling and porosity tests. The membranes exhibited a high average porosity of 85.35%, indicating a fibrous and open-pore architecture produced via the electrospinning process. This high porosity level ensures that fluids can easily diffuse into the inter-fiber voids. Furthermore, the membranes demonstrated a significant average swelling ratio of 507.22%, which confirms the high specific surface area and porous morphology of the nanofibrous structure [6,17]. A direct correlation was observed between the porosity and swelling behavior; higher porosity provides more internal void volume, thereby enhancing the water retention capacity of the membrane. These results indicate that the PLA outer layer possesses the necessary morphological features to support gas exchange while effectively managing fluid interactions at the wound site.

The mechanical performance of the electrospun PLA membranes was evaluated to determine their structural suitability for wound dressing applications. The membranes, with an average thickness of 0.06 mm, exhibited a tensile strength of

4 MPa. During the tensile tests, the samples demonstrated significant elongation before failure, reaching a strain value of 1.348. This high strain indicates a ductile mechanical behavior, where the nanofibers rearrange and distribute the applied load gradually rather than failing abruptly. Such mechanical durability and flexibility are essential for maintaining the integrity of the dressing while conforming to the dynamic contours of the wound site [21, 22]. The surface wettability of the PLA outer layer was characterized by contact angle measurements to evaluate its protective barrier function. The membrane exhibited a high contact angle of 128° , confirming its hydrophobic nature. This hydrophobicity is attributed not only to the intrinsic chemical properties of the PLA but also to the rough, fibrous morphology created by the electrospinning process [17]. In a bilayer system, this hydrophobic outer layer serves as a controlled barrier that protects the wound from external liquid contaminants while simultaneously preserving the moist microenvironment provided by the underlying *Hypericum*-incorporated alginate hydrogel.

The antibacterial performance of the electrospun PLA membranes was evaluated using the agar disk diffusion method against Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*). The membranes exhibited a distinct inhibition zone with a diameter of 1.4 cm against *E. coli*. The fact that the inhibition zone was larger than the membrane diameter indicates that the active components effectively diffused into the agar medium, preventing bacterial growth beyond the immediate contact area. In contrast, no inhibition zone was observed against *S. aureus*. This suggests that while the membrane possesses significant antibacterial efficacy against Gram-negative bacteria, its activity against Gram-positive strains was limited under the tested conditions. These results demonstrate that the PLA-based layer contributes to the wound dressing's protective function by inhibiting specific bacterial growth, thereby reducing the risk of infection at the wound site [17, 22, 23]. Figure 6 shows the antibacterial test.

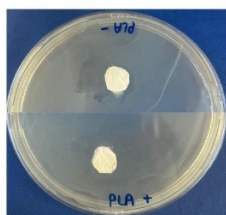


Figure 6. Antibacterial activity test

The mechanical hardness of the hydrogels was measured using a texture analyzer, and the results are

presented in Table 1. The peak force required to compress the samples was recorded as the hardness value. The pure alginate hydrogel (A0) exhibited a hardness of 0.49 kg. The addition of *Hypericum* extract led to a decrease in hardness, with the A2 sample (2% extract) showing the minimum value of 0.36 kg. This trend suggests that the extract components may interfere with the Ca^{2+} cross-linking process, resulting in a more compliant network. However, at higher concentrations (A4), the hardness slightly recovered to 0.44 kg, which may be attributed to a more crowded molecular environment hindering deformation [10, 11].

Table 1. Hardness values of the hydrogel formulations.

Sample code	<i>Hypericum</i> extract concentration (%)	Hardness (kg)
A0	0	0.49
A1	1	0.43
A2	2	0.36
A3	3	0.39
A4	4	0.44

The color properties of the hydrogel samples were evaluated based on the CIELAB (L^* , a^* , b^*) color space, and the results are summarized in Table 2. The incorporation of *Hypericum* extract significantly altered the transparency and optical characteristics of the alginate hydrogels. Pure alginate (A0) exhibited the highest brightness (L^*) value, while a substantial decrease was observed in all extract-incorporated samples. The lowest L^* value was recorded for the A2 formulation, indicating that the extract components interact with the polymer matrix to absorb or scatter light, thereby reducing light transmittance. The a^* (redness) and b^* (yellowness) values reached their peak in the A1 sample, which is directly correlated with the natural pigments found in the *Hypericum* extract [20, 24]. The total color difference (ΔE) for the enriched samples (A1–A4) ranged between 32.15 and 38.59 relative to the control group (A0). The maximum ΔE value was observed in the A2 sample, suggesting that a 2% concentration induces the most significant morphological and chemical color transition. The stabilized ΔE values observed in A3 and A4 indicate that a color saturation point is reached beyond a certain extract concentration.

Table 2. Hypericum-alginate color test

Alginate code	L*	a*	b*	ΔE
A0	55.74	0.8	0.38	2.85
A1	28.4	8.67	14.39	32.15
A2	18.37	4.01	8.7	38.59
A3	22.48	2.95	4.66	33.74
A4	23.09	3.16	6.71	33.49

CONCLUSION

In this study a bioactive bilayer wound dressing consisting of an electrospun PLA outer layer and a *Hypericum* extract-incorporated alginate hydrogel was successfully developed. The PLA layer provided a protective hydrophobic barrier with a 128° contact angle, high porosity (85.35%), and a tensile strength of 4 MPa. The A2 formulation (2% extract) emerged as the optimum inner layer, exhibiting the highest swelling capacity (652%) and improved flexibility. The electrospun PLA outer layer exhibited antibacterial activity against *E. coli*, whereas no inhibition zone was observed against *S. aureus*. FTIR analysis confirmed the stable integration of the extract into the matrix, while mechanical and color tests showed that the extract significantly influenced the structural and optical properties of the hydrogels. Overall, the synthesized bilayer system effectively combines protective barrier functions with high exudate management, making it a promising candidate for advanced wound care applications.

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