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Polylimonene Improves the In Vivo Wound Healing Performance of Sodium Alginate-Based Hydrogels

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ABSTRACT

Efficient wound dressing materials must support tissue regeneration while modulating the oxidative environment. In this study, a photo-crosslinked hydrogel based on methacrylated alginate (AlgMA) containing 3% (w/v) poly(limonene) (PLM) was developed to improve healing outcomes. PLM, a stable and non-volatile antioxidant additive, was incorporated to overcome the limitations of limonene’s volatility and instability. The hydrogel’s efficacy was evaluated in vivo using an excisional wound model in rats and compared to unmodified AlgMA and commercial dressings (Sorbalgon and cotton gauze). Wound contraction was assessed through macroscopic imaging and quantitatively described using mathematical models. The AlgMA/PLM3% hydrogel exhibited the fastest healing dynamics, with a logistic rate constant of 0.63 day^{−1} and a half-maximal healing time of 6.02 days, outperforming AlgMA (7.64 days), Sorbalgon (8.82 days), and gauze (10.78 days). Histological analysis confirmed superior re-epithelialization, granulation tissue density, and dermal organization in the AlgMA/PLM3% group by Day 18. These results demonstrate that the incorporation of PLM into alginate hydrogels enhances wound healing by accelerating closure and promoting tissue organization, positioning PLM as a promising bioactive additive for acute wound care.

1 | Introduction

Wound healing in acute injuries is a complex and dynamic process involving a cascade of overlapping phases: hemostasis, inflammation, proliferation, and remodeling [1, 2]. Each stage is regulated by intricate interactions among cells, cytokines, and extracellular matrix components [1, 3, 4]. Disruptions in this sequence, particularly prolonged inflammation or oxidative stress, can impair tissue repair and lead to chronic wounds [5–7]. Therefore, the selection of appropriate wound dressings plays a key role in modulating the wound environment and supporting physiological healing [8]. Alginate-based hydrogels are widely utilized due to their hydrophilic nature, biocompatibility, and

ability to maintain a moist environment [9–14]. Upon contact with wound exudate, alginate forms a gel that preserves hydration, promotes cellular activity, and facilitates cell adhesion, proliferation, and fibroblast migration during the proliferative phase [15]. Importantly, alginate does not interfere with matrix remodeling, thereby supporting the natural progression of tissue regeneration [16]. Despite these advantages, alginate lacks intrinsic bioactivity, particularly with regard to oxidative stress modulation.

Reactive oxygen species (ROS) are critical signaling molecules in early wound healing, but their excessive accumulation can damage cellular components and delay regeneration [17]. As a

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result, incorporation of antioxidants into wound dressings has emerged as a promising strategy to neutralize ROS, regulate inflammation, and enhance tissue repair [18]. Several antioxidant systems have been explored in hydrogel matrices, including natural polyphenols (such as tannic acid and catechin derivatives) [19, 20], catechol-containing polymers (e.g., polydopamine, dopamine-functionalized chitosan) [21], and vitamin E-loaded formulations [22]. While these materials effectively scavenge free radicals, they often present drawbacks such as strong coloration, poor photostability, limited control of release kinetics, and potential cytotoxicity from oxidation byproducts [5, 23].

In this context, we employed a limonene-derived oligomer (PLM) as a novel, sustainable, and non-volatile antioxidant additive. PLM is synthesized via photoinduced oligomerization of R-(+)-limonene, a renewable terpene abundant in citrus peel waste, resulting in a low-molecular-weight (≈ 1 kDa) oligomer rich in allylic and conjugated double bonds [24, 25]. These structural features enable efficient radical scavenging and oxidative stabilization, while the oligomeric nature enhances miscibility with hydrophilic polymers and prevents rapid volatilization or degradation [26]. PLM can be seamlessly incorporated into

photocrosslinkable hydrogels such as AlgMA, preserving mechanical and optical integrity.

Compared with previously reported antioxidant systems, PLM offers several advantages: it is bio-based and produced under mild, metal-free conditions; and it provides a tunable antioxidant effect linked to its degree of unsaturation [27]. However, its scalability and compositional reproducibility remain challenges that need to be addressed in future translational studies. Despite the growing number of antioxidant-functionalized biomaterials [18, 28, 29], no prior work has evaluated the *in vivo* wound healing performance of limonene-derived oligomers or established mechanistic correlations between their antioxidant behavior, structural properties, and tissue regeneration outcomes.

In the present study, an alginate-methacryloyl (AlgMA)-based hydrogel containing 3% (w/v) PLM was developed to evaluate its wound healing performance *in vivo*, as schematically illustrated in Figure 1. This concentration (3% w/v) was identified as more attractive because it provided the best balance between antioxidant capacity, structural integrity, and cytocompatibility in our previous work. At this level, PLM enhanced

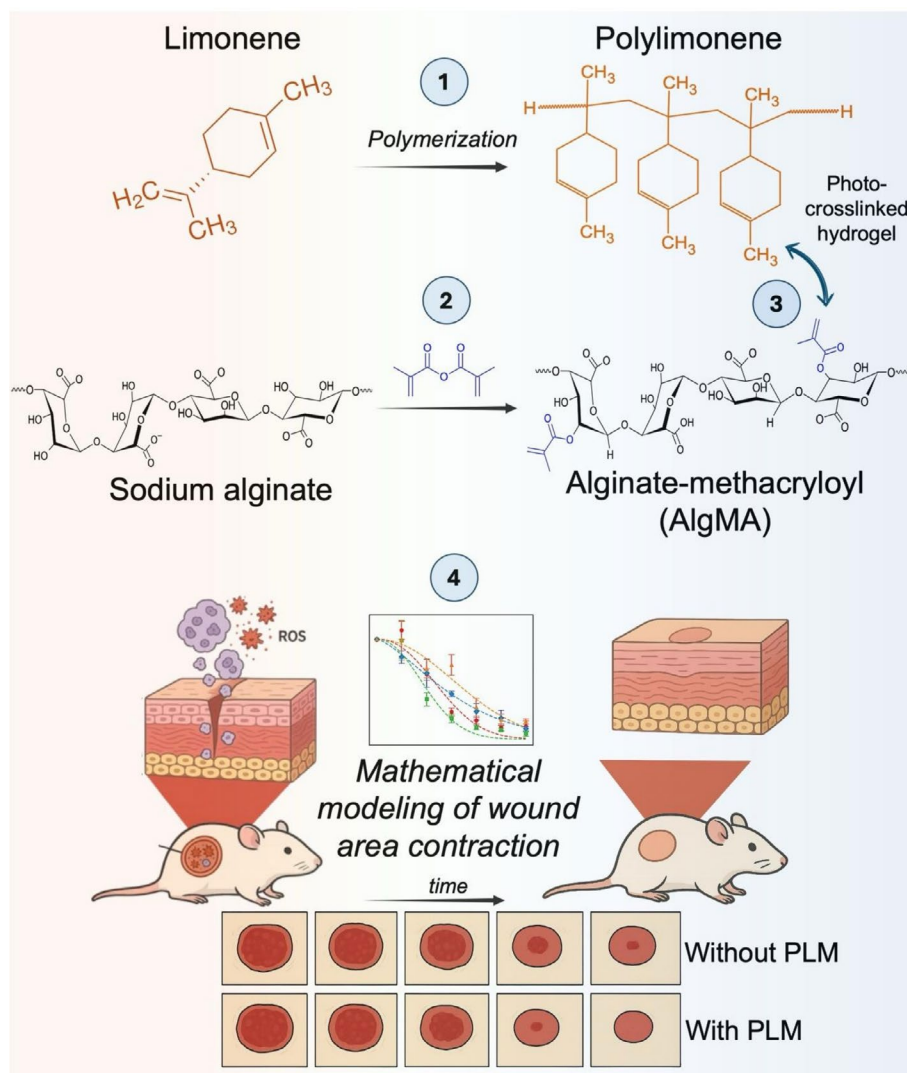


FIGURE 1 | Schematic overview of the synthesis steps and *in vivo* wound healing evaluation, with mathematical modeling applied to provide insights into the healing kinetics. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.70298)]

free radical scavenging activity by approximately 45% compared to AlgMA alone, without compromising crosslinking efficiency or mechanical stability. Higher PLM contents (> 5%) resulted in phase separation and reduced network cohesion, whereas lower concentrations (< 1%) produced limited antioxidant response [26]. Thus, AlgMA/PLM3% formulation was compared to AlgMA (without PLM) and two commercial dressings (Sorbagon and cotton gauze) using an excisional wound model in rodents, which involved removal of the epidermis, dermis, and subcutaneous tissue, enabling detailed analysis of each healing stage, including inflammatory response, granulation tissue formation, re-epithelialization, and matrix remodeling. Wound closure was assessed macroscopically, quantitatively assessed using mathematical models, and further evaluated through histological analysis to determine the impact of PLM incorporation on healing progression. Therefore, this study highlights PLM-incorporated sodium alginate hydrogels as a promising and sustainable alternative for advanced wound dressings, combining effective tissue regeneration with antioxidant protection.

2 | Materials and Methods

2.1 | Chemicals

Sodium alginate (Alg, M/G ratio = 1.522 ± 0.153 , $\overline{M}_n$ of 93.8 kDa), methacrylic anhydride (MA, $\geq 94\%$) and sodium hydroxide (NaOH, analytical grade) were obtained from Sigma-Aldrich (Belgium). Pluronic F127 triblock copolymer [poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide), 80% poly(ethylene oxide), 8400 Da] and 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure I2959, > 98%) were supplied by BASF (Belgium). R-(+)-limonene (> 93%), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, $\geq 99\%$), dimethyl acetamide (DMA, $\geq 99\%$), benzophenone (BP, $\geq 99\%$), and 2,2,2-tribromoethanol (TBE, $\geq 97\%$) were purchased from Sigma-Aldrich (Brazil).

2.2 | Synthesis of Alginate-Based Hydrogels Incorporating Polylimonene

The limonene oligomer (PLM) was synthesized as previously described [25]. Briefly, R-(+)-Limonene (LIM) and dimethyl acetamide (DMA) were mixed in a 1:1 volume ratio in 20 mL glass vials. To this solution, N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA), the catalyst trioxane (TX), and 2,2,2-tribromoethanol (TBE) were added in a molar ratio of 100:5:1:1 (LIM:PMDETA:TX:TBE). The mixture was stirred and purged with nitrogen for 10 min to remove dissolved oxygen, then exposed to UV light (365 nm, 2 mW cm^{-2}) for 6 h at 40°C , as illustrated in Figure 1 (step 1). After irradiation, the product was transferred to Petri dishes and dried at 45°C for 48 h to eliminate unreacted LIM and DMA. The number-average molecular weight ($\overline{M}_n$) of the PLM was determined to be 964 Da based on the analysis of the ^1H NMR spectrum [26]. Parallely, methacryloyl-modified alginate (AlgMA) was synthesized, as indicated in Figure 1 (step 2). A 2% (w/v) sodium alginate solution (1 L) containing 0.0103 mol of hydroxyl groups per gram of polymer was used. Methacrylic anhydride (123 mL;

4 M equivalents, that is, 0.0412 mol/g relative to hydroxyls) was added dropwise. The pH was maintained at ~ 8 throughout the 24 h reaction at 25°C under constant stirring, using a 5 M NaOH solution for adjustment. After the reaction, the mixture was diluted with 1 L of deionized water and dialyzed (12–14 kDa cut-off) for 3 days, with the dialysate replaced twice daily. The dialyzed solution was then transferred to polystyrene Petri dishes (14 cm diameter), frozen at -20°C , and lyophilized for 48 h.

In step 3 (Figure 1), hydrogels were synthesized by combining AlgMA with PLM. Emulsions were first prepared by mixing PLM with an aqueous Pluronic F127 solution at a mass ratio of 1:1 (F127/PLM), followed by sonication in an ultrasound bath for 15 min. To produce the hydrogel films, 5% (w/v) AlgMA solutions were prepared containing 3% PLM (w/w relative to AlgMA), resulting in two sample types: AlgMA (control) and AlgMA/PLM3%. This PLM concentration was selected for its antioxidant activity, sustained release profile, and biocompatibility with fibroblasts in vitro as shown in our previous research [26]. The solutions were deoxygenated with argon for 1 min, and Irgacure I2959 was added to reach a final concentration of 4% (mol/mol) relative to AlgMA methacrylate groups. The mixtures were cast between glass plates separated by a 1 mm silicone spacer and crosslinked under UV-A light (365 nm, 10 mW cm^{-2}) for 30 min.

The structural and morphological characterizations of the synthesized AlgMA and AlgMA/PLM hydrogels were thoroughly described in our previous publication [26]. Briefly, ^1H NMR and FTIR analyses confirmed the successful methacrylation of alginate (appearance of vinyl proton peaks at 5.7 and 6.2 ppm and C=C stretch near 1635 cm^{-1}) and the oligomerization of limonene to PLM. HR-MAS NMR further demonstrated > 85% double-bond conversion after photo-crosslinking, and SEM images revealed a highly interconnected porous morphology, suitable for cell infiltration and exudate absorption. To avoid redundancy, these characterization results are not repeated here but are summarized to facilitate reader comprehension of the materials used in this study.

2.3 | In Vivo Wound Healing Assessment

This stage of the research received approval from the Research Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy in Iași (Romania) and complied with established ethical standards for laboratory animal experimentation (Law no. 206/27 May 2004; EU Directive 2010/63/EU—CE86/609/EEC). The experiments were carried out at the Advanced Research and Development Center for Experimental Medicine (CEMEX) of the same university under certificate no. 2/22.09.2017.

The in vivo study was carried out using adult male Wistar rats (6–8 weeks old, 350–400 g). Animals were housed individually in polypropylene cages for 7 days under standardized conditions: temperature at $23^\circ\text{C} \pm 2^\circ\text{C}$, relative humidity between 37% and 60%, and a 12-h light/dark cycle. They were provided with standard pellet feed and had free access to water. After surgical wound induction, the rats were randomly assigned to four

groups and monitored over an 18-day period. The treatments evaluated included AlgMA, AlgMA/PLM3%, a sterile cotton gauze (negative control), and Sorbalgon (a commercial sodium alginate dressing, positive control). All treated areas were protected using a click dressing, following previously established methods [11]. The protocol involved creating a 2 cm-diameter excisional wound, with dressings changed every 3 days. Wounds were photographed and analyzed using a custom Python script to measure wound area, perimeter, and circularity.

Euthanasia and histological evaluations were performed on Day 18, which corresponds to the late proliferative and remodeling phase of cutaneous repair in this model. This interval was selected based on previous reports demonstrating that complete epithelialization and mature granulation tissue typically occur between 14 and 21 days post-injury in Wistar rats [30]. Thus, at the study's conclusion, animals were humanely euthanized to avoid suffering. Rats were fasted and anesthetized with isoflurane 24 h before euthanasia. After final biopsies, euthanasia was performed via intracardiac injection of 1–2 cc potassium chloride (KCl) under anesthesia, ensuring a swift and painless procedure.

2.4 | Mathematical Modeling of Wound Area Contraction

The wound contraction dynamics were modeled by fitting different mathematical functions to the experimental data representing the average wound area over time. Raw data were organized by treatment group and experimental days, and a reference point at Day 0 was added with a fixed initial wound area of 3.14 cm². Five mathematical models were considered to describe the temporal evolution of wound area, $A(t)$, such as linear (Equation 1), logistic (Equation 2), exponential decay (Equation 3), Gompertz (Equation 4), and Weibull (Equation 5) models.

$$A(t) = A_0 + k_{lin} \cdot t \quad (1)$$

in which k_{lin} is the constant linear rate of area change per day.

$$A(t) = A_0 \cdot \{1 + \exp[k_l \cdot (t - t_0)]\}^{-1} \quad (2)$$

in which k_l is the logistic rate constant, determining the steepness of the curve and t_0 is the inflection point (which means the day of maximum contraction rate).

$$A(t) = A_0 \cdot \exp(k_e \cdot t) \quad (3)$$

in which k_e is the exponential decay constant (a negative value indicating the rate of area reduction).

$$A(t) = A_0 \cdot \exp[-b \cdot \exp(-k_g \cdot t)] \quad (4)$$

in which b is a scaling parameter that controls the initial lag phase of contraction and k_g is the Gompertz rate constant, influencing how quickly the contraction accelerates after the lag.

$$A(t) = A_0 \cdot \exp[-(k_w \cdot t)^\beta] \quad (5)$$

in which k_w is the scale parameter, related to the time scaling of contraction and β is the shape parameter, which modulates the curvature and acceleration of wound contraction.

In all cases, the initial area A_0 was fixed at 3.14 cm², based on the average observed wound area at Day 0. Parameter estimation for each model was performed using nonlinear regression via the Levenberg–Marquardt algorithm implemented in the *curve_fit* function from the *SciPy* library in Python. Model fitting was applied to the mean wound area data for each treatment group across the different time points.

2.5 | Histology

Skin samples, including the wound and adjacent tissue, were fixed in 10% buffered formalin for 48 h. Histological processing used paraffin embedding with a Leica TP1020 tissue processor (Leica Microsystems, Germany). Sections of 5 μm thickness were cut using a SLEE CUT 6062 microtome (SLEE Medical, Germany), then deparaffinized and stained with Masson's trichrome (hematoxylin–eosin–methylene blue). Analysis was performed with a Leica DM750 optical microscope equipped with a Leica ICC50 HD camera, and images captured via Leica Application Suite (LAS) software version 4.2. All histology procedures and imaging were standardized across groups.

2.6 | Statistics

Quantitative data were obtained in triplicate and expressed as mean ± standard deviation. Statistical differences among groups were evaluated using one-way ANOVA, followed by Tukey's post hoc test to determine significant pairwise differences ($p < 0.05$), indicated by different letters next to the means. The quality of fit for each model for wound area reduction was evaluated using the coefficient of determination (R^2) and root mean square error (RMSE). For each treatment, the best-fitting model was selected based on the simultaneous highest R^2 value and lowest RMSE. The optimal fitted curves for each treatment were plotted alongside the experimental data to allow for visual and quantitative comparisons across treatments and models.

3 | Results and Discussion

The wound healing progression was first qualitatively assessed across four treatment groups: AlgMA, AlgMA/PLM3%, Sorbalgon, and cotton gauze, and displayed in Figure 2. By Day 3, all wounds exhibited intense redness and minimal contraction, characteristic of the inflammation phase [31]. Notably, wounds treated with AlgMA/PLM3% began showing early signs of reduced inflammation. By Day 6, these wounds also displayed visible contraction at the margins, whereas wounds in the cotton gauze group remained largely unchanged. During the proliferation phase (Days 9–12), the AlgMA/PLM3%-treated animals demonstrated accelerated healing, evidenced by smaller wound areas and drier surfaces.

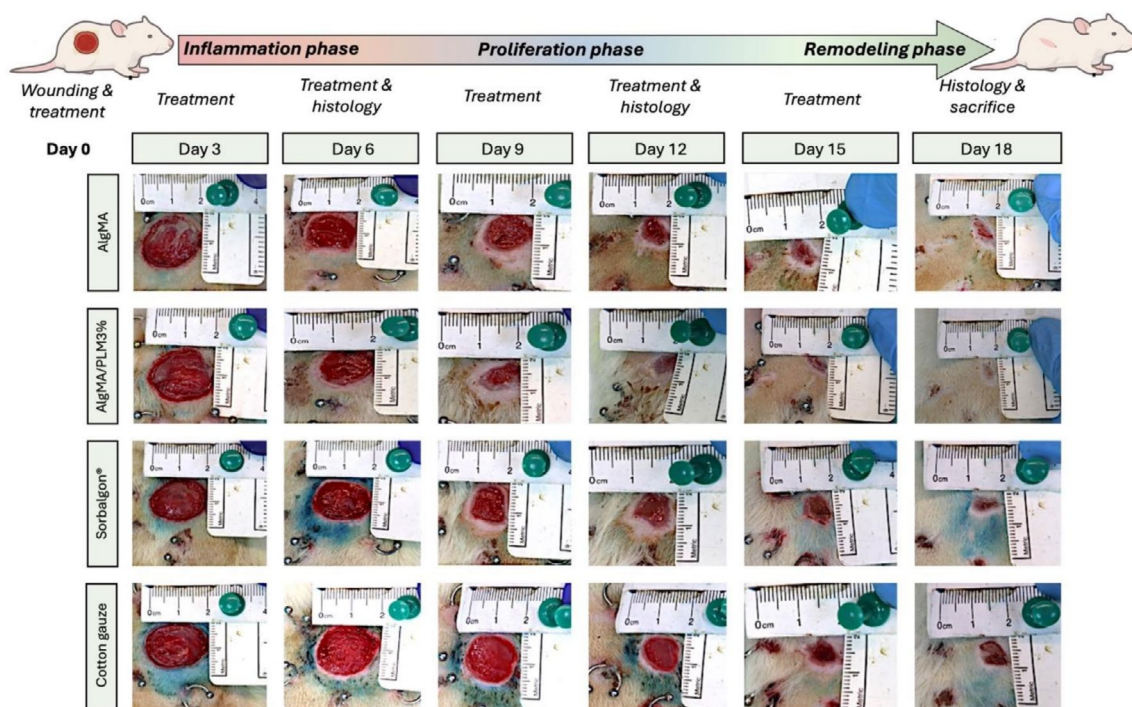


FIGURE 2 | In vivo evaluation of wound healing using AlgMA and AlgMA/PLM3%-based hydrogels compared to commercial wound dressings (Sorbalgon and cotton gauze), with wound images captured every 3 days over an 18-day period. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.70293)]

Sorbalgon and AlgMA treatments supported moderate healing, while cotton gauze-treated wounds continued to appear moist and inflamed. By Day 15, wounds treated with AlgMA/PLM3% showed near-complete closure and signs of re-epithelialization. At Day 18, these samples exhibited the most advanced remodeling, with fully closed wounds and minimal scarring, outperforming the other groups.

The enhanced wound closure observed with AlgMA/PLM3% treatment can be attributed to the potent antioxidant activity of PLM [26, 32], which may have inhibited oxidative stress at the wound site and promoted overall tissue regeneration [33]. Conversely, the reduced performance of Sorbalgon and cotton gauze may be attributed to their clinical limitations. While Sorbalgon is commonly used for moderately exuding wounds, forming a gel upon contact with exudate [15], it may trigger allergic responses [34], reducing its reliability under varied conditions. Cotton gauze, though widely used, is associated with significant drawbacks such as tissue damage during removal, fiber retention in the wound bed (which can lead to granuloma formation), wound drying, and higher infection rates [35, 36], contributing to the less favorable healing outcomes.

Simultaneously, the wound healing progression was quantitatively assessed by fitting the reduction in wound area over time to different mathematical models, including Logistic, Weibull, Exponential, Linear, and Gompertz models. Weibull model provided the best overall fit (Figure 3a and Table 1). These mathematical models allow for a deeper understanding of the healing dynamics and provide parameters that can be correlated with the rate and pattern of wound closure. For instance, in the Logistic model, the AlgMA/PLM3% treatment exhibited a higher rate parameter (0.63 day^{-1}) than

AlgMA (0.53 day^{-1}), Sorbalgon (0.27 day^{-1}) and cotton gauze (0.31 day^{-1}). Mathematically, this result suggests that AlgMA/PLM3% accelerated wound closure more effectively from the early stages of healing. The time to half-maximal healing (t_0) was also shorter for AlgMA/PLM3% (6.02 days) compared to AlgMA (7.64 days), Sorbalgon (8.82 days), and cotton gauze (10.78 days), reflecting a faster onset of tissue regeneration. In terms of model fit, AlgMA/PLM3% outperformed AlgMA ($R^2=0.95$ vs. 0.93 ; $\text{RMSE}=0.27$ vs. 0.32) and showed better predictive accuracy than the commercial dressings. Although Sorbalgon and gauze had similar R^2 values (0.98 and 0.94 , respectively), their higher inflection times and lower rate parameters confirm a slower and less efficient healing process, particularly in the early and intermediate phases of wound repair.

The Weibull model, identified as the best fit for the wound healing data, was used to simulate healing kinetics, as shown in Figure 3a for comparison with the experimental results. This analysis further emphasized the advantage of AlgMA/PLM3%, which exhibited a higher shape parameter (0.14) compared to AlgMA (0.11), Sorbalgon (0.09), and cotton gauze (0.08), confirming a more rapid acceleration phase of healing. The scale parameter for AlgMA/PLM3% was $2.12 \text{ day}^{-1/\beta}$, slightly lower than that of AlgMA ($2.26 \text{ day}^{-1/\beta}$), but significantly higher than Sorbalgon ($1.42 \text{ day}^{-1/\beta}$), suggesting a more sustained healing trajectory without early plateauing. Again, cotton gauze presented the poorest healing kinetics by the lowest shape parameter. Figure 3b highlights three key time points from the wound healing assays, where statistical analysis corroborated the trends predicted by the Logistic and Weibull models. By Day 6, the AlgMA/PLM3% group showed a significantly smaller wound area compared to the

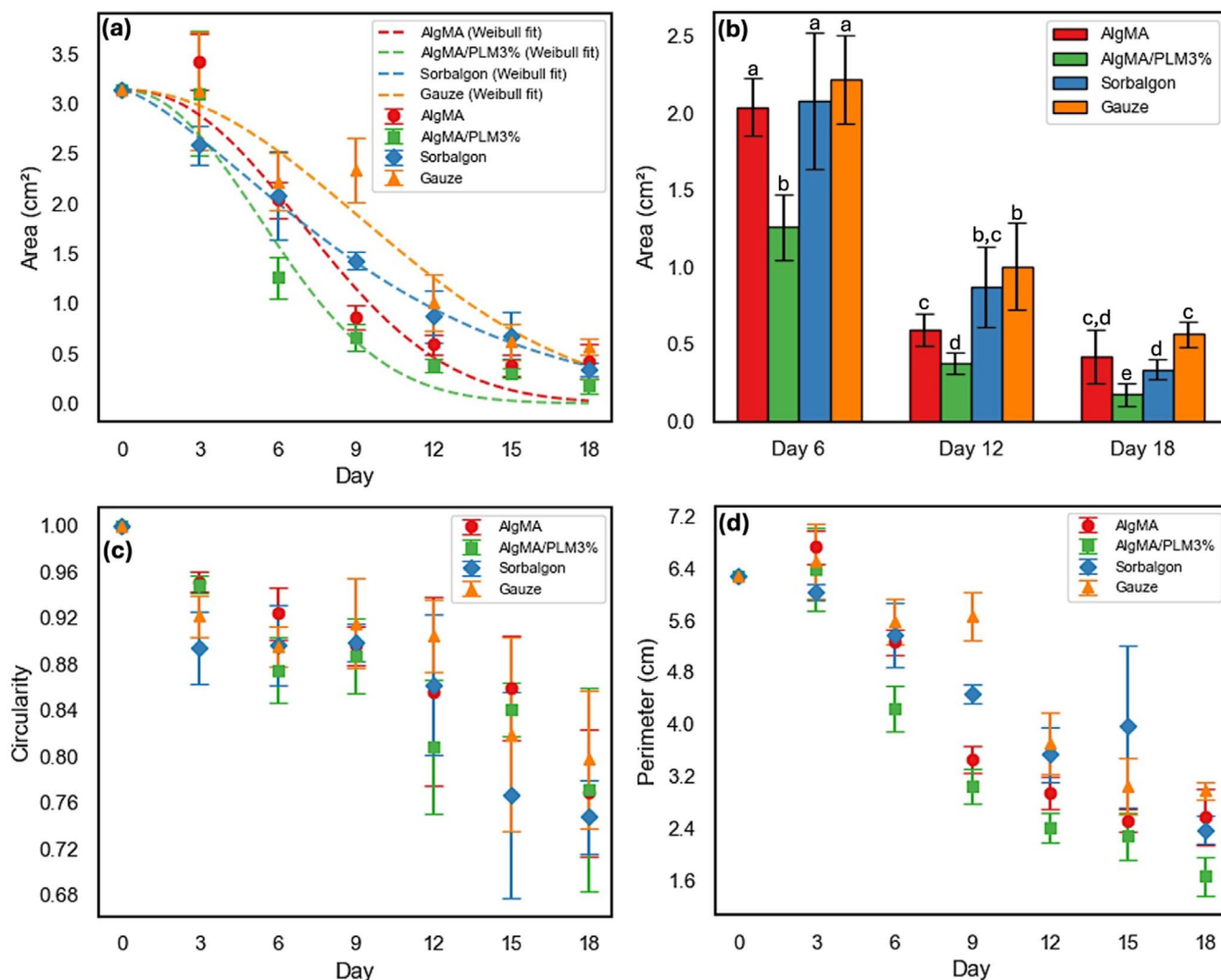


FIGURE 3 | (a) Wound area reduction over time with Weibull model fitting curves overlaid for each treatment group, with error bars representing standard deviation of the mean ($n=7$); (b) comparison of wound area at Days 6, 12, and 18, in which bars with different letters indicate statistically significant differences between treatments at each time point ($p < 0.05$); (c) circularity, and (d) perimeter measurements of the wounds over time. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

other treatments ($p < 0.05$). In contrast, wounds treated with AlgMA alone, Sorbalgon, or cotton gauze showed no statistically significant differences among them ($p > 0.05$). This trend persisted through Days 12 and 18, with AlgMA/PLM3% consistently being the only treatment associated with a statistically smaller wound area, confirming its superior performance throughout the healing process.

Complementarily, Figure 3c depicts the wound circularity, a shape descriptor where 1.0 represents a perfect circle, indicating symmetrical healing. All treatments started near 1.0 and showed a slight decline. AlgMA/PLM3% and AlgMA generally preserved better circularity than Sorbalgon, suggesting a more uniform wound contraction. However, variability increases with time, likely due to differences in wound closure mechanisms and biological variability. Figure 3d shows a progressive decline in wound perimeter for all treatments. Again, AlgMA/PLM3% shows a steeper decrease, supporting its effectiveness in promoting tissue contraction. Gauze- and Sorbalgon-treated samples demonstrate slower reductions, consistent with the area data.

Histological analysis (Figure 4) provided further evidence supporting the enhanced wound healing observed macroscopically, particularly in the AlgMA/PLM3% group. On Day 6, all groups displayed incomplete re-epithelialization and an inflammatory infiltrate characteristic of the early inflammatory phase. However, the AlgMA/PLM3% group showed denser granulation tissue and better organized cellular architecture at the wound edge compared to the other treatments. In contrast, Sorbalgon and cotton gauze samples presented disorganized tissue with pronounced inflammatory infiltrates and minimal epithelial advancement. By Day 12, tissue regeneration was more evident in all groups but at varying degrees. The AlgMA/PLM3% group exhibited a more continuous and thicker neoepidermis, along with early dermal matrix remodeling and vascularization, suggesting a transition into the proliferative phase [2]. AlgMA and Sorbalgon showed moderate epithelial coverage but lacked the same dermal organization, while cotton gauze-treated wounds remained inflamed with evident disruption in tissue continuity and thinner epidermal layers.

TABLE 1 | Estimated parameters, coefficient of determination (R^2), and root mean square error (RMSE) for each mathematical model fitted to wound area data by treatment group.

Treatment	Model	Parameter 1	Parameter 2	R^2	RMSE
AlgMA	Logistic	$k_l = 0.53 \text{ day}^{-1}$	$t_0 = 7.64 \text{ days}$	0.93	0.32
	Weibull	$\beta = 0.11$	$k_w = 2.26 \text{ day}^{-1/\beta}$	0.94	0.30
	Exponential	$k_e = -0.10 \text{ day}^{-1}$	—	0.83	0.50
	Linear	$k_{lin} = -0.18 \text{ cm}^2 \text{ day}^{-1}$	—	0.85	0.47
	Gompertz	$b = 0.02$	$k_g = -0.49 \text{ day}^{-1}$	0.90	0.38
AlgMA/PLM3%	Logistic	$k_l = 0.63 \text{ day}^{-1}$	$t_0 = 6.02 \text{ days}$	0.95	0.27
	Weibull	$\beta = 0.14$	$k_w = 2.12 \text{ day}^{-1/\beta}$	0.96	0.25
	Exponential	$k_e = -0.14 \text{ day}^{-1}$	—	0.88	0.41
	Linear	$k_{lin} = -0.20 \text{ cm}^2 \text{ ay}^{-1}$	—	0.82	0.51
	Gompertz	$b = 0.05$	$k_g = -0.43 \text{ day}^{-1}$	0.92	0.34
Sorbalgon	Logistic	$k_l = 0.27 \text{ day}^{-1}$	$t_0 = 8.82 \text{ days}$	0.98	0.14
	Weibull	$\beta = 0.09$	$k_w = 1.42 \text{ day}^{-1/\beta}$	1.00	0.06
	Exponential	$k_e = -0.09 \text{ day}^{-1}$	—	0.97	0.18
	Linear	$k_{lin} = -0.17 \text{ cm}^2 \text{ ay}^{-1}$	—	0.98	0.15
	Gompertz	$b = 0.13$	$k_g = -0.18 \text{ day}^{-1}$	0.95	0.21
Gauze	Logistic	$k_l = 0.31 \text{ day}^{-1}$	$t_0 = 10.78 \text{ days}$	0.94	0.25
	Weibull	$\beta = 0.08$	$k_w = 2.08 \text{ day}^{-1/\beta}$	0.94	0.25
	Exponential	$k_e = -0.07 \text{ day}^{-1}$	—	0.83	0.42
	Linear	$k_{lin} = -0.15 \text{ cm}^2 \text{ ay}^{-1}$	—	0.91	0.32
	Gompertz	$b = 0.06$	$k_g = -0.22 \text{ day}^{-1}$	0.92	0.30

On Day 18, the differences among treatments became more pronounced. The AlgMA/PLM3% group displayed a nearly complete regeneration of the skin, including a well-formed epidermis and a more organized dermal layer, closely resembling normal tissue. Sorbalgon also showed evidence of healing, but with delayed epidermal stratification and irregular collagen deposition. AlgMA-treated wounds showed partial maturation of the dermis, while cotton gauze-treated tissues continued to exhibit signs of incomplete remodeling, including residual inflammatory cells and disorganized epidermal layers. Taken together, these histological findings corroborate the superior wound contraction and healing kinetics observed in the AlgMA/PLM3% group, supporting the notion that the incorporation of PLM at 3% not only accelerates closure but also promotes more organized tissue regeneration. This reinforces its potential as an effective bioactive dressing for wound management. Moreover, the enhanced performance of AlgMA/PLM3%, especially in the later stages of healing, suggests that the incorporation of PLM amplifies the regenerative properties of the base hydrogel. This may explain its continued efficacy during the remodeling phase, where many treatments lose effectiveness [1, 3], and aligns with the previous qualitative and quantitative descriptions of this study.

The superior regenerative performance of the AlgMA/PLM3% hydrogel can be directly associated with its structural and

mechanical characteristics described in our previous study [26]. In that work, the hydrogel exhibited a storage modulus of approximately 3–4 kPa, a gel fraction close to 90%, and a swelling degree near 1500%, indicating an optimized crosslinking density that ensures both structural integrity and high exudate absorption capacity. The interconnected porous network facilitates cell migration, vascular ingrowth, and efficient oxygen and nutrient diffusion. These features create a mechanically compliant yet supportive environment, reproducing the viscoelastic microarchitecture of dermal tissue. Combined with the controlled release of PLM, which mitigates oxidative stress and inflammation [27], this structural profile explains the accelerated epithelialization, improved collagen organization, and reduced scar formation observed in vivo. Altogether, these findings highlight that the enhanced healing response results from the synergistic interaction between the optimized mechanical/morphological design of the AlgMA matrix and the bioactive antioxidant functionality of PLM.

While the results obtained highlight the therapeutic promise of AlgMA/PLM3% hydrogels, several factors must be considered for clinical translation. The synthesis of PLM from R-(+)-limonene is a straightforward photoinduced oligomerization carried out under mild conditions [25], which favors scalability and reproducibility compared with more complex polymerizations used for polyphenol- or catechol-based antioxidant

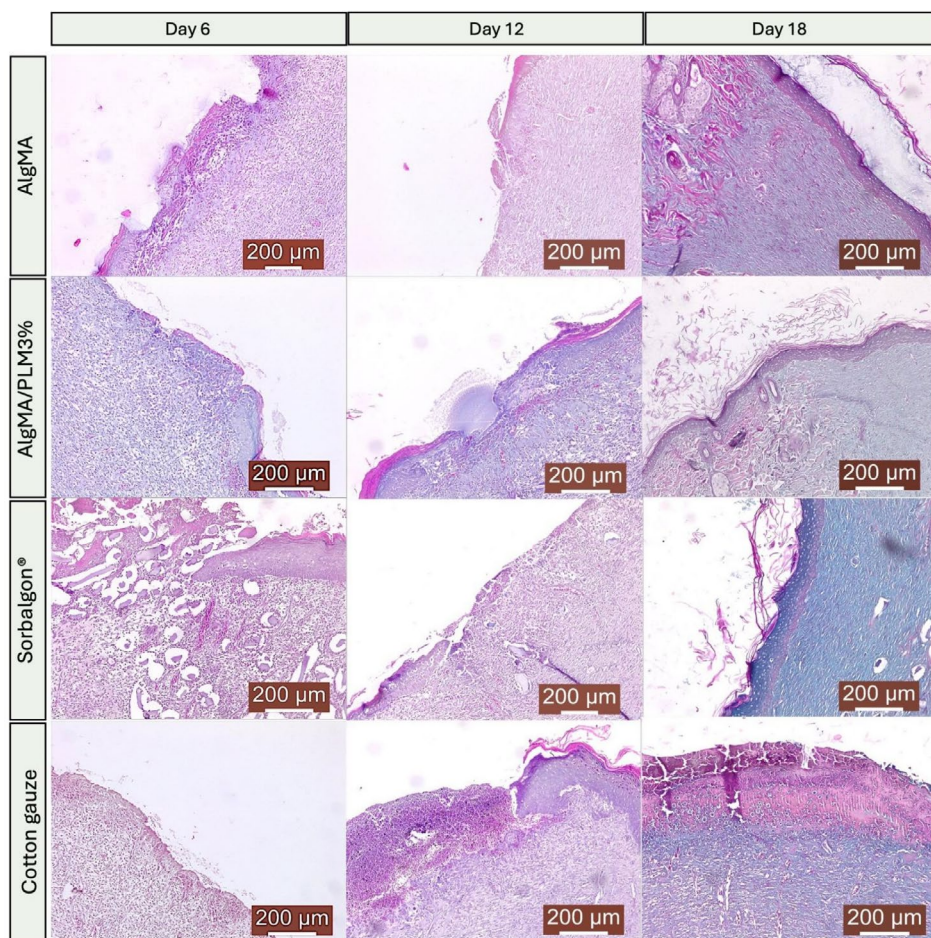


FIGURE 4 | Masson's trichrome staining of back wound tissues collected on Days 6, 12, and 18 from animals treated with different wound dressings. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.70293)]

systems (e.g., polydopamine, tannic acid-functionalized polymers) [19, 20]. However, process optimization will be required to ensure consistent molecular weight distribution and batch-to-batch reproducibility when scaled to pilot production. Another challenge lies in regulatory validation and long-term biocompatibility testing, particularly for degradation products that may form under physiological conditions [37]. Nonetheless, the renewable origin of limonene, its established safety profile in food and cosmetic applications [38], and the material's demonstrated bioactivity position the AlgMA/PLM3% hydrogel as a sustainable and clinically relevant alternative to synthetic antioxidant polymers. Future studies should explore automated photoreactor synthesis and GMP-compliant purification routes to advance PLM-based biomaterials toward preclinical development.

4 | Conclusion

The combined qualitative, quantitative, and histological analyses demonstrated that the incorporation of 3% PLM into the AlgMA hydrogel matrix significantly enhanced wound healing outcomes compared to both the pure AlgMA and commercial dressings. AlgMA/PLM3% promoted earlier resolution of inflammation, faster wound contraction, and more complete tissue regeneration, as reflected in smaller wound areas, improved wound geometry, and superior re-epithelialization. The Weibull

model provided the best fit for wound area data, quantitatively confirming the accelerated and sustained healing associated with this formulation. Histological findings further substantiated these observations, revealing more organized dermal architecture and advanced remodeling in the AlgMA/PLM3% group. The enhanced healing performance is attributed to the antioxidant activity of PLM, which modulated oxidative stress and supported tissue regeneration throughout all phases of the healing process. These results reinforce the potential of AlgMA/PLM3% as a promising bioactive dressing for improved wound management.

Author Contributions

Roni  rik Pioli Vieira: conceptualization (lead), data curation (lead), funding acquisition (lead), investigation (lead), writing – original draft (lead). **Guilherme Frey Schutz:** methodology (supporting). **Oana-Maria Chirliu:** methodology (supporting). **Aurelian-Sorin Pasca:** methodology (supporting). **Lenuta Profire:** methodology (supporting), writing – review and editing (supporting). **Sandra Van Vlierberghe:** funding acquisition (supporting), investigation (supporting), writing – review and editing (supporting).

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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 from the corresponding author.

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