Poly(Lactide) Nonwoven Fabric with Iron Coating and Its Biological Properties

Zdzisława Mrozińska 1, Małgorzata Świerczyńska 1,2, Michał Juszczak 1,3, Katarzyna Woźniak 3, and Marcin H. Kudzin 1,*

1 Łukasiewicz Research Network—Łódź Institute of Technology, Marii Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland; zdzislawa.mrozinska@lit.lukasiewicz.gov.pl (Z.M.)
2 Institute of Polymer and Dye Technology, Faculty of Chemistry, Łódź University of Technology, Stefanowskiego 16, 90-337 Łódź, Poland
3 Department of Molecular Genetics, Faculty of Biology and Environmental Protection, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland; katarzyna.wozniak@biol.uni.lodz.pl
* Correspondence: marcin.kudzin@lit.lukasiewicz.gov.pl; Tel.: +48-42-6163121

Abstract: The study investigated the biological properties of a composite material composed of poly(lactide) (PLA) and iron (Fe) produced by sputtering iron onto melt-blown poly(lactide) nonwoven fabrics. The research aimed to thoroughly understand the structure and properties of these materials and their potential applications in biomedicine. We conducted comprehensive chemical and structural analyses using techniques such as microscopic analysis, flame atomic absorption spectrometry (FAAS), and Brunauer–Emmett–Teller (BET) surface area analysis to precisely determine the properties of PLA-Fe materials. Additionally, we evaluated their impact on blood coagulation processes by measuring activated partial thromboplastin time (aPTT) and prothrombin time (PT). We also performed biological analyses on human peripheral blood mononuclear cells (PBM cells) including cell viability and DNA damage. Our results clearly demonstrate that PLA-Fe materials do not significantly influence blood coagulation mechanisms, as they only slightly prolong aPTT time and have no effect on PT. This suggests their potential in biomedical applications. Our results indicate the absence of cyto- and genotoxic properties of PLA-Fe materials against normal blood cells. In conclusion, the research findings suggest that the novel poly(lactide) and iron-sputtered nonwoven fabrics are promising tools in the field of biomedicine, offering potentially innovative therapeutic solutions for the treatment of wounds and injuries.

Keywords: blood coagulation; cell viability; composite; DNA damage; iron; melt-blown; poly(lactic acid); nonwoven; sputtering

1. Introduction

The melt-blown process is a single-phase, efficient, and solvent-free technology that directly transforms solid polymer into nonwoven fabric [1–3]. During this process, the polymer is melted in an extruder, where polymer pellets are mechanically sheared by a continuously rotating screw at high temperature. The molten polymer is then passed through a die with numerous small-diameter holes, forming fibers. This production method endows the fibers with unique physical and chemical properties, making them attractive for various applications [4–6]. Compared to traditional fabrics, melt-blown nonwoven fabric is characterized by a simplified production process, lower costs, and excellent air permeability. Despite these advantages, the melt-blown nonwoven fabric retains a large surface area, small pores, excellent barrier properties, and mechanical strength [7–9].

Nonwoven fabrics, commonly utilized in dressings, implants, and scaffolds, possess an open structure that is optimal for exudate drainage, significantly reducing the likelihood of infection [10]. The three-dimensional, fibrous architecture of these materials mimics natural tissue, creating an appropriate matrix for tissue regeneration [11,12]. Owing to
their high porosity, nonwoven fabrics support the development of new tissues within their matrix, facilitating efficient nutrient transport and waste elimination. As a result, these fabrics are effectively employed in the regeneration of different tissue types, such as skin and epithelial tissues [13–16].

The wound-healing process is complex and involves several phases: hemostasis, inflammation, proliferation, and maturation, each influenced by multiple factors [17]. To expedite this process, appropriate conditions must be provided at the wound site. Modern dressings must meet several key criteria: they should be nontoxic, hypoallergenic, maintain a moist environment while removing excess exudate, possess antibacterial properties or be impermeable to bacteria, enable gas exchange, and be economically viable. The physicochemical properties of polylactide (PLA), such as biocompatibility, biodegradability, and mechanical strength, partially fulfill these requirements, making PLA a suitable material for modern wound dressings [18–29]. PLA can be synthesized both by direct polycondensation of lactic acid and by ring-opening polymerization of lactide [30–33]. This aliphatic, semi-crystalline polyester is characterized by ease of processing and good thermoplasticity [34–39]. PLA has a glass transition temperature in the range of 55–60 °C and a melting point of around 175 °C [40,41], which makes it suitable for processing methods such as melt blowing. Moreover, PLA is recognized by the FDA as a nontoxic material, ensuring its safety for human health and the environment [42,43]. Through a wide range of processing methods, including extrusion, film casting, injection molding, fiber spinning, electrospinning, foaming, and micro- and nanofabrication techniques, PLA-based biomaterials can be formed into various shapes and sizes. This processing versatility is crucial for expanding PLA applications in the biomedical field [44–46].

Scientific research is uncovering mechanisms by which iron can enhance coagulation processes and reduce the rate of fibrinolysis. Ferric chloride has been identified as an agent that increases both the rate of clot formation and the final strength of clots. Combinations of iron with other molecules exhibit complex prothrombotic kinetic effects. Parallel ultrastructural analyses have revealed specific cross-linking and tarnishing patterns of fibrin polymers in plasma exposed to ferric chloride, with each substance imparting unique properties to the resulting structures. The results of these studies suggest that iron influences key processes of coagulation and fibrinolysis, leading to the formation of clots that initiate more rapidly, develop more dynamically, exhibit greater strength, and are less susceptible to enzymatic degradation [47,48].

Recent studies have concentrated on improving the properties of PLA by incorporating a range of fillers and additives. Notably, the introduction of iron (Fe) particles into PLA matrices has emerged as a promising approach for enhancing material performance, though this field remains less investigated compared to other filler systems. Jiang et al. [49] investigated the production of biodegradable scaffolds for bone repair using additive manufacturing. They used PLA as the primary material and incorporated two types of iron-based particles: 316L stainless steel and pure iron. Utilizing fused filament fabrication (FFF) technology, they fine-tuned the printing parameters to create scaffolds with precise pore structures. The study revealed that both PLA/Fe and PLA/316L scaffolds exhibited improved compressive and flexural moduli, increased compressive strength, and greater resistance to compressive fatigue due to the addition of iron-based particles. Furthermore, the PLA/iron scaffolds showed superior in vitro degradation rates and better cytocompatibility compared to the PLA/316L scaffolds. These findings indicate that PLA-iron composite scaffolds have significant potential for bone defect repair applications.

Bakina et al. [50] engineered PLA/Fe$_3$O$_4$ composites that demonstrated improved mechanical properties and biocompatibility in comparison to pure PLA. In another study, Basheer et al. [51] developed a hybrid composite by incorporating 1 wt% carbon fiber and 1 wt% graphene into PLA. Mechanical tests indicated that the 3D-printed hybrid PLA material exhibited a significantly higher tensile strength of 63 MPa, as opposed to 31 MPa for the non-3D-printed material. This enhancement in mechanical properties suggests the potential of this material for use in the automotive industry and consumer products.
Additionally, Zinc oxide (ZnO) has been shown to enhance the mechanical, degradable, and antibacterial characteristics of PLA composites. Boro et al. [52] created PLA/ZnO nanocomposites through solvent casting and explored the effect of ZnO nanoflower content on PLA. They produced two types of PLA nanocomposites containing ZnO nanorods and nanoflowers, with FE-SEM confirming the flower-like morphology of ZnO. The tensile strength of PLA/0.5ZnO reached about 32.75 MPa, surpassing pure PLA, while its elastic modulus peaked at 912 MPa and elongation at break was about 4.8%. Increasing ZnO concentration beyond 0.5 wt% reduced mechanical properties, underscoring the importance of nanofiller dispersion in PLA. The novelty of this research is its thorough investigation of PLA-iron composites, particularly focusing on their impact on blood coagulation. This study seeks to fill a notable gap in the literature by providing an in-depth analysis of how iron particles influence the functional properties of PLA. Additionally, the research examines new applications of PLA-iron composites, specifically studying their effects on the viability of PBM cells and analyzing any potential DNA damage in these cells.

In this study, melt-blown PLA nonwoven fabrics were functionalized through the application of magnetron sputtering with iron particles. Magnetron sputtering enabled the precise application of thin metal layers onto the PLA surface, which is particularly advantageous due to its ecological, economical, and waste-free nature [53–57]. The combination of polylactide (PLA) and iron results in new materials with significant potential in the biomedical sector, particularly for wound treatment. Iron can enhance coagulation properties [48,58,59], contributing to the effective inhibition of blood flow from wounds, while PLA ensures biocompatibility, biodegradability, and adequate mechanical strength [22,60,61]. The composites produced can significantly improve the effectiveness of modern medical dressings by accelerating hemostasis and supporting tissue regeneration. The synergy between iron and PLA enables these materials to find broad therapeutic applications, offering both mechanical support and the bioactivity necessary for effective wound healing. Given the valuable properties of both iron and PLA, we investigated the impact of these composites on the blood plasma coagulation process, a key factor in initiating wound healing, to better understand their effects on blood coagulation mechanisms. Another aspect of determination of biological properties was analysis of influence post-incubation mixtures obtained after incubation with PLA and PLA-Fe on cell viability and DNA damage in PBM cells served as model of normal human cells. Post-incubation mixtures were selected as most appropriate way to test the influence of PLA in cells. This research allowed us to assess the potential of PLA–Fe composite as an active material that supports hemostasis and wound regeneration processes, potentially enhancing the effectiveness of modern medical dressings.

2. Materials and Methods

2.1. Materials

Granular poly(lactic acid) (PLA) polymer, identified as Ingeo™ Biopolymer 3251D, was purchased from NatureWorks LLC (Minnetonka, MN, USA). This polymer featured a melt flow rate (MFR) of 30–40 g/10 min (measured at 190 °C with a 2.16 kg load) and had a melting point between 160 and 170 °C. These granules were used to create the nonwoven fabric samples. The iron target was obtained from Testbourne Ltd. (Basingstoke, UK) with 99.99% purity. Lyophilized human blood plasma and reagents for aPTT (Dia-PTT) and PT (Dia-PT) measurements, along with a 0.025 M CaCl₂ solution, were obtained from Diagon Kft (Budapest, Hungary). A coagulometer (model K-3002 OPTIC, KSELMED, Grudziadz, Poland) was used for the measurements. Prior to conducting the measurements, the reagents were prepared according to the manufacturer’s instructions. Low-melting-point (LMP) and normal-melting-point (NMP) agarose, phosphate-buffered saline (PBS), 4′,6-diamidino-2-phenylindole (DAPI), resazurin sodium salt, dimethyl sulfoxide (DMSO), and hydrogen peroxide (H₂O₂) were purchased from Sigma-Aldrich (St. Louis, MO, USA).
2.2. Methods

2.2.1. Method for Producing PLA Nonwoven Fabric

To produce poly(lactic acid) nonwoven fabric, the melt-blowing technique was employed utilizing a single-screw laboratory extruder from Axon (Åstorp, Sweden), equipped with a nozzle featuring 30 holes, each with a diameter of 0.25 mm. The technological parameters utilized for the production of poly(lactic acid) nonwoven samples are outlined in Table 1.

Table 1. Parameters of the melt-blowing process for poly(lactic acid) nonwoven fabric production.

<table>
<thead>
<tr>
<th>Extruder Temperature [°C]</th>
<th>Air Flow Rate [mln3/godz.]</th>
<th>Mass per Unit Area of Nonwoven Fabrics [g/m]</th>
<th>Yield [g/min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1 240 Zone 2 260 Zone 3 260 Head 260 Air Heater 260</td>
<td>8–9</td>
<td>200</td>
<td>6</td>
</tr>
</tbody>
</table>

2.2.2. Modification of Magnetron Sputtering of Poly(lactide) Nonwoven Fabrics with Iron

To modify PLA nonwoven samples, the direct current (DC) magnetron sputtering process was employed using a system from P.P.H. Jolex sc. (Częstochowa, Poland). Detailed parameters of the direct current magnetron sputtering process used to modify the nonwoven fabrics are presented in Table 2 below.

Table 2. Data regarding the direct current magnetron sputtering process used to modify PLA nonwoven fabrics.

| Target 99.99% Iron | Power Discharge 0.4; 0.8 [kW] | Power Density 0.60 [W/cm²] | Working Pressure 2.1 × 10⁻³ [mbar] | Working Atmosphere Argon | Distance between Sample and Target 15 [cm] | Deposition Time 20; 40 [min] | Sputtered Sample Size 60 × 20 [cm × cm] |

2.2.3. Flame Atomic Absorption Spectroscopy (FAAS)

The iron content in the composite samples was measured using a single-module Magnum II microwave mineralizer from Ertec (Wrocław, Poland). For the analysis, a Thermo Scientific Thermo Solar M6 atomic absorption spectrometer (LabWrench, Midland, ON, Canada) was employed. This device included a titanium burner with a diameter of 100 mm and utilized coded single-element hollow cathode lamps. Background correction during the measurements was achieved with the help of a D2 deuterium lamp.

2.2.4. Microscopy Analysis

The structure of the poly(lactide) nonwoven fabrics was examined using a Keyence VHX-7000N digital microscope from Osaka, Japan. Observations were carried out at magnifications between 500× and 5000×.

For further detailed analysis, a Tescan Vega 3 scanning electron microscope (SEM) was employed, produced by Tescan Analytics in Brno, Czech Republic. This SEM is equipped with an energy-dispersive X-ray spectroscopy (EDS) system from Oxford Instruments, based in Abingdon, UK. SEM imaging was performed under high vacuum conditions with a probe beam energy set at 15 keV, and magnifications ranged from 5000× to 10,000×. The EDS system’s performance was assessed using primary standards provided by Oxford Instruments, following the guidelines of ISO 15632:2012 [62].

2.2.5. Biochemical and Hematological Assessments: Activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT)

Human plasma was first frozen and then lyophilized before being dissolved in deionized water. For each test, 1 mg of the sample was added to 200 µL of the reconstituted plasma, mixed thoroughly, and incubated at 37 °C for 15 min before centrifugation.

To measure activated partial thromboplastin time (aPTT), a Dia-PTT reagent was prepared, containing kaolin, cephalin, and a 0.025 M calcium chloride (CaCl₂) solution.
Each sample was treated with 50 µL of plasma and 50 µL of the Dia-PTT reagent, and then incubated in a coagulometer at 37 °C for 3 min. Following this, 50 µL of 0.025 M CaCl$_2$ was added to start the aPTT measurement. For prothrombin time (PT) testing, 100 µL of plasma was incubated at 37 °C for 2 min, after which 100 µL of Dia-PTT reagent was introduced to initiate the test. The Dia-PTT reagent, which includes rabbit brain tissue thromboplastin, calcium ions, and a preservative, was well mixed before each test to ensure consistent results. Both aPTT and PT measurements were carried out using a K-3002 OPTIC coagulometer. Each test was performed in triplicate, and results are reported as the mean with a standard deviation of approximately 2%.

2.2.6. Determination of Biological Properties

Preparation of Fabrics Used to Assess Biological Properties

To analyze the influence of poly(lactide) on cells, fragments of poly(lactide) and poly(lactide)-Fe fabrics were cut into 1 cm$^2$ pieces (1 × 1 cm), seeded on a 6-well plate, and incubated with 3 mL of RPMI medium at 37 °C in 5% CO$_2$ for 24 h. After that, the post-incubation mixtures were filtered with a 0.2 µm filter to obtain aseptic conditions. Then, the poly(lactide) post-incubation mixtures were added to the cells in a 1:1 ratio to analyze their influence on cell viability and DNA damage.

Cell Culture

Peripheral blood mononuclear cells (PBMCs) were isolated from a leukocyte-buffy coat provided by healthy, nonsmoking donors at the Blood Bank in Łódź, Poland [63]. The isolation process started by mixing the buffy-coat blood with phosphate-buffered saline (PBS) in equal volumes. This mixture was then subjected to density-gradient centrifugation with Lymphosep (Cytogen, Zgierz, Poland) at 2200 RPM for 20 min, ensuring minimal acceleration and deceleration. After centrifugation, the PBMCs were washed three times with 1% PBS via centrifugation. The isolated cells were then suspended in RPMI 1640 medium. The research protocol was authorized by the University of Łódź Committee for Research on Human Subjects (17/KBBN-UL/III/2019).

Cell Viability Resazurin Assay

The cell viability resazurin assay was performed using the method described by O’Brien et al. [64]. Resazurin salt powder was dissolved in a sterile PBS buffer. Post-incubation mixtures were added to PBM cells in the count of 5 × 10^4, and then incubated for 24 and 48 h at 37 °C in 5% CO$_2$. The negative control was RPMI1640 medium prepared in the same manner as post-incubation mixtures. The experiment included a positive control, cell samples incubated with hydrogen peroxide (H$_2$O$_2$) at 500 µM for 15 min at 37 °C. Next, 10 µL of resazurin salt was added to each well and the plates again were incubated at 37 °C in 5% CO$_2$ for 2 h. After that, fluorescence was measured with HT microplate reader BioTek Synergy HT (Agilent Technologies, Inc., Santa Clara, CA, USA) using $\lambda_{ex} = 530/25$ and an $\lambda_{em} = 590/35$ nm. The effects of poly(lactide) post-incubation mixtures were quantified as the percentage of control fluorescence.

DNA Damage

Poly(lactide) post-incubation mixtures were added to PBM cells in the count of 7.5 × 10^4, and then incubated for 24 and 48 h at 37 °C in 5% CO$_2$. The negative control was RPMI1640 medium prepared in the same manner as post-incubation mixtures. The experiment included a positive control, cell samples incubated with hydrogen peroxide (H$_2$O$_2$) at 25 µM for 15 min on ice. The PBM cells after treatment with poly(lactide) post-incubation mixtures were washed and suspended in the RPMI medium.

The comet assay was conducted under alkaline conditions following a modified procedure [65]. A freshly prepared cell suspension in 0.75% low-melting-point (LMP) agarose, dissolved in phosphate-buffered saline (PBS), was layered onto microscope slides previously coated with 0.5% normal-melting-point (NMP) agarose. Cells were then lysed
for 1 h at 4 °C in a solution containing 2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, and 1% Triton X-100, with a pH of 10. After lysis, the slides were transferred to an electrophoresis unit where the DNA was allowed to unwind for 20 min in a solution of 300 mM NaOH and 1 mM EDTA, maintaining a pH above 13. Electrophoresis was performed in a solution of 30 mM NaOH and 1 mM EDTA, also with a pH greater than 13, at 4 °C (with the running buffer temperature kept below 12 °C) for 20 min at an electric field strength of 0.73 V/cm (28 mA). Following electrophoresis, the slides were rinsed with water, allowed to air dry, stained with 2 µg/mL DAPI, and covered with coverslips. The entire procedure was carried out under minimal light or in the dark to avoid further DNA damage.

Comet images were captured at a magnification of 200× using an Eclipse fluorescence microscope (Nikon, Tokyo, Japan), which was coupled with a COHU 4910 video camera (COHU, Inc., San Diego, CA, USA). The setup included a UV-1 A filter block and was connected to a personal computer running the Lucia-Comet v. 6.0 image analysis software (Laboratory Imaging, Prague, Czech Republic). From each sample, fifty comet images were randomly chosen, and the average percentage of DNA in the comet tail was calculated to represent DNA damage.

3. Results and Discussion
3.1. Preparation of Nonwoven Fabrics from Poly(lactide) and Modification by Magnetron Sputtering

Poly(lactic acid) (PLA) nonwoven fabrics were fabricated via melt blowing employing a laboratory single-screw extruder. Initially, PLA is subjected to melting within the extruder, where polymer granules undergo mechanical shearing facilitated by a high-temperature rotating screw. The extruder comprises three primary zones: feeding (1), passage (2), and dosing (3). Subsequently, the molten polymer is forced through a matrix containing numerous small apertures, leading to fiber formation. These fibers are then elongated by high-speed hot air on a collector surface (refer to Figure 1).

![Figure 1. Schematic of the PLA-blowing process.](image)

Unlike traditional nondegradable melt-blown nonwoven fabrics, polyactic acid (PLA) melt-blown nonwoven fabrics are renewable and biodegradable, providing notable environmental advantages at the end of their service life. Despite these ecological benefits, PLA melt-blown nonwoven fabrics typically have limitations in terms of strength and toughness, which may restrict their practical use. This study presents a simple yet effective approach
to enhance the mechanical properties of PLA melt-blown nonwoven fabrics [66]. PLA-based nonwoven fabrics are distinguished by their softness, lightweight, and flexibility. Additionally, their significant surface area and high porosity make them highly suitable for a variety of applications, including air filtration, tissue engineering, regenerative medicine, wound care, and drug delivery systems [67,68].

The obtained PLA nonwoven fabric underwent magnetron sputtering (MS) technology, facilitating the precise deposition of thin iron coatings (Figure 2). Through this methodology, we engineered a composite material comprising polylactide and iron (PLA-Fe(kW/t)).

Figure 2. Picture of the poly(lactic acid) nonwoven (A) and PLA-Fe[0.8kW/40] composite (B).

3.2. Determination of Iron Content

The analysis of iron concentration in poly(lactide)-iron fiber samples was conducted using flame atomic absorption spectrometry (FAAS), and the results are presented in Table 3. The magnetron power plays a crucial role during the coating of polylactide fibers with iron. Higher sputtering power (0.8 kW) enables the attainment of the maximum quantity of iron ions, which is essential for the durability and efficiency of the coating. The results showed the uniformity of iron coverage. Durable iron coatings have the potential to be applied in the production of dressings and medical devices, contributing to the enhancement of hygiene, health, and safety.

Table 3. Results of iron content determination in the tested samples.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Power Discharge [kW]</th>
<th>Sp.T. /a [min]</th>
<th>Iron Bulk Concentration /b,c [g/kg]</th>
<th>MBC /d [mol/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLA-Fe[0.4kW/20]</td>
<td>0.4</td>
<td>20</td>
<td>2231</td>
<td>0.04</td>
</tr>
<tr>
<td>PLA-Fe[0.4kW/40]</td>
<td>0.4</td>
<td>40</td>
<td>18,832</td>
<td>0.34</td>
</tr>
<tr>
<td>PLA-Fe[0.8kW/20]</td>
<td>0.8</td>
<td>20</td>
<td>4422</td>
<td>0.08</td>
</tr>
<tr>
<td>PLA-Fe[0.8kW/40]</td>
<td>0.8</td>
<td>40</td>
<td>28,341</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Sp.T. — duration of sputtering deposition in minutes. Results derived from Equation (1). b,c Data were obtained from three independent measurements and reported as the average with a standard deviation of approximately 2%. d MBC—molal bulk concentration, where MFe = 55,845 mg/mol. PLA-Fe (MBC) — PLA nonwoven fabric combined with iron material after a specific sputtering deposition time (Sp.T.) and characterized by the iron molal bulk concentration (MBC).

3.3. Microscopy of PLA-Fe Samples

The microscopy images reveal subtle differences in the surface morphology of the analyzed PLA and PLA-Fe(kW/t) fiber samples, attributable to the iron magnetron sputtering process (Figure 3). Unmodified samples exhibit an evenly distributed fiber structure with a smooth surface. The magnetron sputtering process (PLA → PLA-Fe(kW/t)) induces subtle alterations in surface morphology. In PLA-Fe(kW/t) samples, slightly elongated fibers are discernible...
compared to PLA samples, while maintaining a similar overall fibrous structure. Microscopic observations further indicate the absence of visible iron agglomerates on the fiber surface in PLA-Fe\textsuperscript{(kW/0)} samples, suggesting a homogeneous deposition of the iron layer across the entire surface (PLA-Fe\textsuperscript{(0.8kW/40)}). No damage in the structure of individual PLA nonwoven fibers was observed after Fe-sputtering modification. The presence of such a layer may significantly influence the physical and chemical properties of the material, particularly in its interactions with the environment and potential applications in biomedicine.

**Figure 3.** Microscope images of PLA and PLA-Fe\textsuperscript{(kW/0)}. Magnification: 50×; 1000×.

The scanning electron microscopy (SEM) analysis was conducted using magnifications of ×5000 and ×10,000 to examine the samples in detail. For the PLA (Polylactic Acid) sample, SEM images at ×5000 magnification revealed a relatively smooth and uniform surface. This level of magnification allowed for the observation of the general morphology of the PLA, showing no significant surface irregularities or inclusions, which suggested a pure and homogeneous polymer matrix. When observed at ×10,000 magnification, the SEM images provided an even finer resolution, capturing minute surface details and confirming the uniformity of the polymer structure. The high magnification images demonstrated that the PLA sample was free of contaminants and structural anomalies.

In the case of the PLA–Fe\textsuperscript{(0.8kW/40)} sample, SEM analysis at ×5000 magnification demonstrated that the iron does not exhibit significant clustering or agglomeration, indicating a relatively even dispersion. At ×10,000 magnification, the SEM provided a more detailed view of the interaction between the iron particles and the PLA matrix. This
high-resolution imaging confirmed that, while iron particles are present, they are dispersed without noticeable aggregation. However, at the same magnification used for the PLA samples, the PLA–Fe\(^{(0.8\text{kW}/40)}\) images revealed that the fibers are fractured (Figure 4, sample: PLA–Fe\(^{(0.8\text{kW}/40)}\), magnifications: \(\times10,000\)). Fiber breakage and damage of the PLA-Fe\(^{(0.8\text{kW}/40)}\) material occurred during the 40 min high-energy magnetron sputtering process; this occurrence was confirmed in our previous studies [69]. The surfaces of the PLA–Fe\(^{(0.8\text{kW}/40)}\) samples, like the PLA samples, were found to be smooth and uniform overall, with no substantial clusters of iron or significant agglomerations observed. This thorough SEM analysis provided critical insights into the surface topography. The fiber diameter in the unmodified (PLA) and modified (PLA–Fe) samples ranged from 4 to 9 µm.

Figure 4. Cont.
Figure 4. Microscope images of PLA and PLA-Fe(0.8kW/40) samples: (A) PLA (magnification: 5000×); (B) PLA (magnification: 10,000×); (C) PLA-Fe(0.8kW/40) (magnification: 5000×); (D) PLA-Fe(0.8kW/40) (magnification: 10,000×).

The analysis involved using a scanning electron microscope (SEM) combined with energy-dispersive X-ray spectroscopy (EDS) to examine the samples. This approach allowed for a thorough evaluation of the materials’ elemental composition. The EDS technique provided detailed information by measuring the X-ray spectra, where the position and intensity of the peaks indicated the presence and concentration of various elements. The results, shown in Figure 5, verified the presence of iron in the samples, consistent with data obtained from flame atomic absorption spectroscopy (FAAS).

Figure 5. Experimental data from energy-dispersive X-ray spectroscopy (EDS).
3.4. Analysis of Surface Properties and Pore Volume

Table 4 details the findings regarding the specific surface area and overall pore volume for both untreated polylactide nonwoven fabric (PLA) and polylactide nonwoven-iron composites (PLA-Fe\(^{(kW/t)}\)). Notable variations in these properties were detected following the iron deposition via magnetron sputtering. The specific surface area of the nonwoven fabric dropped from 0.9470 to approximately 0.7028 after coating with iron. This decrease is attributed to the iron filling the pores on the fabric’s surface. Similarly, the total pore volume reduced from 2.868 \(\times\) 10\(^{-3}\) for the uncoated PLA fabric to about 2.008 \(\times\) 10\(^{-3}\) for the iron-coated samples, indicating a decline in porosity due to the iron deposition. The average pore diameter (D) results are related to the specific surface area (SSA): samples with smaller pores (D) have a larger specific surface area (SSA) (Table 4). Additionally, increasing the magnetron power resulted in a thicker iron layer, leading to more pronounced changes in both the specific surface area and total pore volume. These observations highlight the substantial effects of magnetron sputtering on the polymer nonwoven fabric, including reduced porosity and alterations in surface characteristics.

Table 4. Analysis of the specific surface area, overall pore volume, and average pore diameter for both treated polylactide (PLA) nonwoven fabric and PLA-Fe composites.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Specific Surface Area (SSA) [m(^2)/g]</th>
<th>Total Pore Volume (TPV) [cm(^3)/g]</th>
<th>Average Pore Diameter (D) [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>0.9470</td>
<td>2.868 (\times) 10(^{-3})</td>
<td>14.92</td>
</tr>
<tr>
<td>PLA-Fe(^{(0.4kW/20)})</td>
<td>0.9082</td>
<td>2.605 (\times) 10(^{-3})</td>
<td>15.47</td>
</tr>
<tr>
<td>PLA-Fe(^{(0.4kW/40)})</td>
<td>0.7659</td>
<td>2.128 (\times) 10(^{-3})</td>
<td>18.03</td>
</tr>
<tr>
<td>PLA-Fe(^{(0.8kW/20)})</td>
<td>0.8681</td>
<td>2.554 (\times) 10(^{-3})</td>
<td>16.59</td>
</tr>
<tr>
<td>PLA-Fe(^{(0.8kW/40)})</td>
<td>0.7028</td>
<td>2.008 (\times) 10(^{-3})</td>
<td>18.45</td>
</tr>
</tbody>
</table>

Figure 6 depicts the nitrogen (N\(_2\)) adsorption and desorption profiles for both untreated PLA nonwoven fabric and PLA-Fe\(^{(kW/t)}\) composites. The profiles follow a Type III isotherm shape, as defined by IUPAC, which indicates minimal interaction between the material and the nitrogen gas. This shape suggests that the nitrogen molecules predominantly adhere to specific sites on surfaces that are either nonporous or have micropores [65,70–72]. The adsorbate reaches a saturation point at a certain value of saturation pressure (p/p\(_0\) = 1), signifying a multilayer adsorption mechanism across the entire pressure range. Type III isotherms are commonly linked to materials with either nonporous or microporous characteristics [73,74]. Analysis of the graphs reveals an exponential augmentation in the amount of adsorbed nitrogen with increasing pressure. Initially, the rate of increase is gradual at low relative pressure, while at pressures approaching p/p\(_0\) = 1, the amount of adsorbed gas experiences a rapid escalation [53]. The appearance of H3-type hysteresis loops indicates that the material has slotted pores that can trap some of the adsorbate even at lower pressures. As pressure increases, there is a swift rise in the amount of adsorbate, typical of substances with such slotted pore structures [75–77].
Figure 6. N₂ adsorption–desorption isotherms for the (a) PLA; (b) PLA-Fe\((0.4\text{kW}/20)\); (c) PLA-Fe\((0.4\text{kW}/40)\); (d) PLA-Fe\((0.8\text{kW}/20)\); (e) PLA-Fe\((0.8\text{kW}/40)\) samples.
3.5. Effect of PLA-Fe Samples on Blood Plasma Coagulation: aPTT and PT Measurement

The variations observed in activated partial thromboplastin time (aPTT) following the deposition of iron on PLA can be attributed to interactions between the deposited iron particles and blood coagulation factors (Figure 7). Pure PLA nonwovens exhibit an aPTT of approximately 37 s. The incorporation of iron, however, increases aPTT, with values ranging from approximately 42 to 50 s, depending on sputtering power and deposition duration. This extended aPTT with iron suggests potential effects on the intrinsic pathway of coagulation. Elevated aPTT values, particularly with increased iron deposition, imply that iron might alter the surface properties of PLA and interact with clotting factors, potentially modifying their activity and leading to prolonged clot formation times. In contrast, minimal changes in prothrombin time (PT) suggest that the extrinsic coagulation pathway is largely unaffected by iron deposition (Figure 8).

![Figure 7](image_url1)  
**Figure 7.** Effect of the tested iron-coated poly(lactide) nonwoven fabrics on aPTT: PLA, PLA-Fe\(^{0.4kW/20}\), PLA-Fe\(^{0.4kW/40}\), PLA-Fe\(^{0.8kW/20}\), PLA-Fe\(^{0.8kW/40}\), and C (control) samples. Results are presented as mean (×), median (horizontal line), range (bars), and interquartile range (box).

![Figure 8](image_url2)  
**Figure 8.** Effect of the tested iron-coated polylactide nonwoven fabrics on PT: PLA, PLA-Fe\(^{0.4kW/20}\), PLA-Fe\(^{0.4kW/40}\), PLA-Fe\(^{0.8kW/20}\), PLA-Fe\(^{0.8kW/40}\), and C (control) samples. Results are presented as mean (×), median (horizontal line), range (bars), and interquartile range (box).
Based on the analysis of aPTT and PT, a relationship between the iron content in the composite (PLA-Fe) and the effect on blood clotting processes has been demonstrated. Higher iron content (PLA-Fe\(_{(0.4kW/40)}\), PLA-Fe\(_{(0.8kW/40)}\)—Fe content: 18,832 and 28,341 g/kg, respectively) in the composite materials results in longer coagulation pathway times compared to the unmodified poly(lactide) nonwoven sample (PLA). The PLA-Fe\(_{(0.4kW/20)}\) sample (Fe content: 2231 g/kg) demonstrates a moderate increase in aPTT while showing minimal changes in PT relative to other samples, indicating a balanced effect on coagulation pathways.

These findings underscore the potential for PLA-Fe composites in biomedical applications where controlled blood coagulation is critical. Nonetheless, further research is necessary to fully understand the clinical significance and practical implications of these effects on blood coagulation and overall biocompatibility in biomaterial applications.

3.6. Effect of PLA-Fe Samples on the Viability of PBM Cells

We used the resazurin reduction assay to determine cell viability after incubation with PLA and PLA-Fe post-incubation mixtures. This assay is based on the application of an indicator dye to measure oxidation-reduction reactions, which principally occur in the mitochondria of live cells. The nonfluorescent dark blue dye (resazurin) becomes fluorescently pink at 570 nm and fluorescently red at neutral pH (resorufin) when reduced by metabolically active cells. In this experiment, we used 500 µM hydrogen peroxide (H\(_2\)O\(_2\)) as a positive control. We observed a decrease in cell viability to the 37% of control (\(p < 0.001\)) after incubation with H\(_2\)O\(_2\). We showed that incubation of PBM cells with PLA and PLA-Fe post-incubation mixtures did not decrease cell viability after 24 and 48 h (Figure 9) incubation. Our results indicate the absence of cytotoxic properties of PLA and PLA-Fe materials against PBM cells. These results suggest that iron present in PLA fabric does not induce a Fenton reaction [78]. During Fenton’s reaction, extremely reactive hydroxyl radicals may be formed and exhibit destructive potential towards biomolecules such as DNA, proteins, and lipids [79,80].

![Figure 9. Effect of poly(lactide) PLA and poly(lactide)-Fe PLA-Fe\(_{(0.4kW/20)}\), PLA-Fe\(_{(0.4kW/40)}\), PLA-Fe\(_{(0.8kW/20)}\), PLA-Fe\(_{(0.8kW/40)}\) post-incubation mixtures on PBM cell viability after 24 and 48 h incubation. Results are presented as mean result from 6 repeats. Error bars denote SD; *** \(p < 0.001\).](image)

3.7. Effect of PLA-Fe Samples on the DNA Damage in PBM Cells

The comet assay in the alkaline version is a sensitive and simple method for determining the level of DNA damage, including single- and double-strand breaks and alkali-labile sites in living cells [81]. We observed severe DNA damage in PBM cells incubated with 25 µM H\(_2\)O\(_2\) (positive control). In the case of PLA and PLA-Fe post-incubation mixtures, we did not observe an increase in DNA damage after 24 and 48 h (Figure 10) incubation.
Moreover, we present pictures of comets (Figure 11) that show no DNA damage in the case of post-incubation mixtures, whereas hydrogen peroxide-induced severe DNA damage is visible.

Figure 10. Effect of poly(lactide) PLA and poly(lactide)-Fe PLA-Fe(0.4kW/20), PLA-Fe(0.4kW/40), PLA-Fe(0.8kW/20), PLA-Fe(0.8kW/40) post-incubation mixtures on PBM cell DNA damage after 24 and 48 h incubation. Results are presented as mean results from 100 comets. Error bars denote SEM; *** p < 0.001.

Our studies have shown that PLA and PLA-Fe do not cause DNA damage in PBM cells. However, the Fe²⁺/Fe³⁺ redox system may give these materials specific chemical and catalytic properties. Our research is consistent with other studies that have shown the lack of cytotoxic and genotoxic effects of Fe-containing materials. Recently, it was demonstrated that Mg₀.₁⁻γ-Fe₂O₃(mPEG-silane)₀.₅ nanoparticles did not produce any DNA strand breaks or oxidative DNA damage in lung cancer A549 cells and normal bronchial epithelial BEAS-2B cells. Similarly, no cytotoxicity of the tested nanoparticles was found, although these nanoparticles slightly increased reactive oxygen species in both cell types studied [81]. Other studies conducted on endothelial cells (EA.hy926) and smooth muscle cells (A7r5) showed that the biocompatibilities and antibacterial properties of Zn-3Cu alloy are significantly improved by the alloying of trace Fe. Furthermore, its hemocompatibility is not adversely affected, which indicates that Zn-Cu-Fe alloy can be a promising vascular stent candidate material [82,83].

Fe-containing materials such as nanoparticles and polymers are intensively researched for their application as drug delivery systems, antibacterial therapeutics, biocatalysts, imaging agents, and biosensors in the biomedical field [84]. The new poly(lactide) nonwoven materials we developed and tested, produced through melt blowing and subsequently subjected to iron magnetron sputtering, expand the range of potential biomedical applications of Fe materials to include the treatment of wounds and injuries.
Figure 10. Effect of poly(lactide) PLA and poly(lactide)-Fe PLA-Fe(0.4kW/20), PLA-Fe(0.4kW/40), PLA-Fe(0.8kW/20), PLA-Fe(0.8kW/40) post-incubation mixtures on PBM cell DNA damage after 24 and 48 h incubation. Results are presented as mean results from 100 comets. Error bars denote SEM; ***p < 0.001.

Figure 11. Effect of post-incubation mixtures on PBM cell DNA damage after 24 h incubation: medium (A); 25 µM hydrogen peroxide (B); poly(lactide) PLA (C); poly(lactide)-Fe PLA-Fe(0.4kW/20) (D); PLA-Fe(0.4kW/40) (E); PLA-Fe(0.8kW/20) (F); and PLA-Fe(0.8kW/40) (G).

4. Conclusions

The newly developed poly(lactide) nonwoven materials, fabricated via melt blowing and subsequently subjected to iron magnetron sputtering, were extensively studied to gain a comprehensive understanding of their structural and biochemical properties. Chemical and structural analyses were performed using techniques such as flame atomic absorption spectrometry (FAAS) and specific surface area (BET) analysis, allowing for a detailed examination of the chemical composition and surface characteristics of PLA-Fe(kW/t) materials. Additionally, we assessed the biochemical properties of these materials, with a primary focus on their influence on blood coagulation processes.
By measuring activated partial thromboplastin time (aPTT) and prothrombin time (PT), we evaluated the effect of these new materials on blood plasma coagulation mechanisms. Our findings indicate that PLA-Fe(kW/t) materials exert only a minor impact on blood clotting, resulting in negligible changes in aPTT. This minimal alteration in aPTT is favorable, as it suggests that the materials do not significantly disrupt the coagulation cascade, thereby preserving the natural blood clotting function. Such characteristics are beneficial for biomedical applications, especially in wound-dressing contexts.

The minimal effect on coagulation suggests that PLA-Fe(kW/t) materials are appropriate for use in dressings and other wound-care products, providing effective protection without adversely affecting blood clotting. Moreover, the absence of cytotoxic and genotoxic effects on PBM cells further supports their potential for safe application in medical contexts. In conclusion, the results indicate that these poly(lactide) iron-modified non-woven fabrics may serve as valuable biomedicine tools, offering innovative solutions for wound and injury management while ensuring compatibility with the body’s natural healing processes.

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References


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