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Electrospun Fibers of Ecovio® Polymer Blends with Antimicrobial Tea Tree Essential Oil: Enhanced Chemical and Biological Properties

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Abstract: This study presents, for the first time, the development of fibers with favorable properties for biodegradable wound dressings made from the Ecovio® (EC) polymer blend, composed of poly(lactic acid) (PLA) and poly(butylene adipate-co-terephthalate) (PBAT), incorporated with tea tree essential oil (TTE). TTE presented antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, achieving minimal inhibitory concentrations of 15 and 7.5 mg/mL, respectively. The TTE was mixed with EC in a binary chloroform and formic acid (85/15 v/v) mixture, leading to homogeneous and wettable fibers by electrospinning. The EC/TTE fibers were characterized confirming the TTE in the fibers. The tests showed that TTE (0.5, 1.0 or 1.5 mL) improved the polymer blend electrospinnability. TTE (1.5 mL or 75 w% concerning the EC) lead to homogeneous fibers with an average diameter of 278 nm. TTE (75 w%) increased the wettability of the EC fibers from 120±2° to 69±1°. Preliminary bacterial adhesion and proliferation assays demonstrated that the EC/TTE fibers have anti-adhesive activity e seems to be more toxic against *P. aeruginosa* compared to *S. aureus* after 24 hours of incubation. Compatibility tests with human blood indicated that the EC/TTE fibers accelerate blood coagulation. The EC/TTE fibers exhibit promising chemical and biological properties (*in vitro*) for the development of wound dressings.

Keywords: electrospinning; *Melaleuca alternifolia*; BLOOD coagulation

1. Introduction

Essential oils are typically found in plants but can also be synthesized by bacteria and insects [1]. They consist mainly of terpenoids but may include oxygenated compounds such as alcohols, ketones, aldehydes, esters, and phenolics [2]. These compounds possess unique biological properties, such as boosting the immune system, providing aromatherapeutic benefits that promote emotional well-being when diffused [3], and exhibiting anti-inflammatory [4,5], analgesic [6], antioxidant [4,5,7], antifungal [3], and bactericidal [3,8] activities.

Among various essential oils, tea tree essential oil (TTE) or *Melaleuca alternifolia* essential oil has gained significant attention primarily due to its antimicrobial, anti-inflammatory, and wound-healing properties [9]. *Melaleuca alternifolia*, commonly known as the tea tree, is a member of the Myrtaceae family and is native from Australia and the Indian Ocean Islands [10,11]. The primary

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product extracted from this plant is TTE, which has a yellowish color and distinctive odor. Australian Aboriginals have used this oil for thousands of years for its bactericidal and antifungal effects [12].

The TTE has been shown to promote rapid wound healing in clinical studies [10,11]. In one study, wounds in 10 patients healed faster when treated with TTE compared to conventional treatment that did not use the essential oil. The results demonstrated a significantly reduced healing time for all participants treated with TTEO [9,13]. The oil's wound-healing properties are primarily attributed to its anti-inflammatory, antioxidant, and antimicrobial capabilities [9]. It is recommended for treating skin lesions, insect bites, burns, acne, and nail fungal infections [14].

TTE is a complex mixture of many compounds, with around 100 components. The primary constituents include terpinen-4-ol, γ-terpinene, and α-terpinene [12,14,15]. Terpinen-4-ol is the major component and is used for treating skin blemishes and relaxing muscles and joints. For TTE to exhibit antiseptic activity, the Australian committee stipulates that its composition should contain less than 15% cineole and more than 30% terpinen-4-ol [16]. Terpinen-4-ol, which constitutes 30-40% of the oil, is responsible for its antimicrobial activity by damaging the bacterial cell membrane [12].

However, the direct topical use of TTE has some disadvantages. When applied to the skin, it can cause contact dermatitis (an allergic skin rash), erythema, erythema multiforme-like eruption, linear IgA bullous disease, systemic hypersensitivity reactions, and anaphylaxis [17–19]. Additionally, its high commercial value poses a challenge, as approximately 300 kg of plant leaves are required to produce just 1 L of the oil. Incorporating TTE into polymeric materials can help mitigate or even prevent contact dermatitis and other side-effects promoted by the topical application of the TTE [20]. It is highlighted the development of materials incorporated with TTE, such as films with poly(lactic acid) (PLA) [20] and electrospun fibers with poly(vinyl pyrrolidone) [21]. These materials can retain the excellent biological properties (e.g., antimicrobial and anti-inflammatory activities [20,21]) of the TTE while reducing side-effects by avoiding its direct topical application.

To produce fibers, the electrospinning technique has garnered significant interest from researchers in the fields of tissue engineering, biomedicine, pharmaceuticals, and functional materials. This is due to its ability to produce fibers with a high surface area-to-volume ratio and average diameters in the submicrometric and nanometric range [22–24]. These fibers can be used in tissue engineering, primarily for wound protection (wound dressings) and tissue repair (scaffolds), as they mimic the properties of some native tissues and organs that present fibrillar proteins, like collagen and elastin [25].

Wound dressings aid in the healing process without necessarily accelerating it. They play a crucial role in maintaining homeostasis, preventing bleeding, remove excess of exudates and protecting the wound against bacteria [26,27]. Wound dressings should not interact directly with the wound site and must be easily removable to prevent further damage. Their primary function is to shield the wound from external agents and contamination, promoting oxygenation, exudate removal, blood coagulation, and inhibiting bacterial adhesion and proliferation. Unlike scaffolds, wound dressings need to be periodically replaced on the injured area [28–31].

In this context, the commercial and biodegradable blend Ecovio® (EC) fiber show great potential for such applications although it does not have antimicrobial activity [32–34]. EC is compostable and composed of hydrophobic and biodegradable polyesters, including PLA and poly(butylene adipate-co-terephthalate) (PBAT) [33,35]. Currently, BASF manufactures plastic materials based on the EC blend for uses such as organic waste packaging, sutures, and prostheses [36].

This study presents the preparation of electrospun EC fibers with TTE into their composition. The fibers were prepared with different concentrations of TTE in a chloroform and formic acid mixture (85/15 v/v). EC/TTE fibers were characterized by Scanning Electron Microscopy (SEM), X-ray Photoelectron Spectroscopy (XPS), contact angle measurements, mechanical properties, and Differential Scanning Calorimetry (DSC). Adhesion and proliferation assays of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) indicated that the fibers possess antimicrobial activity primarily against *P. aeruginosa*. This study demonstrates for the first time that TTE incorporated into EC fibers can induce blood coagulation within just 15 minutes of contact. These properties make EC/TTE fibers promising candidates for evaluation as protective agents (wound

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dressings) for skin wounds, as they can prevent prolonged bleeding and protect the wounds against bacterial deposition and proliferation.

2. Materials and Methods

2.1. Materials

The commercial blend Ecovio® (EC), composed of 55% poly(butylene adipate-co-terephthalate) (PBAT) and 45% poly(lactic acid) (PLA), was generously donated by BASF (São Paulo, Brazil). The solvents chloroform (99%) and formic acid (98%) were purchased from Sigma-Aldrich (Brazil). Tea tree essential oil (TTE), with a density of 0.8818 g/mL, was obtained from dōTERRA. Its chemical composition includes terpinen-4-ol, ranging from 20% to 60% terpinene, with up to 55% in its composition, and alpha-pinene, ranging from 1% to 10%.

2.2. Electrospun Solutions, Electrospinning, and Fiber Preparation

The fibers were prepared following the experimental procedure reported in the literature [33] with modifications. EC solutions (5 mL) were obtained in a chloroform/formic acid mixture (85/15 v/v) at 23°C. The TTE was added to the pre-prepared EC solution and thoroughly mixed by magnetic stirring. Table 1 presents the chemical composition of the electrospun solutions.

Table 2. Chemical solutions used in the electrospinning.

Solutions	EC (% w/v)	TTE (mL)	TTE (% m/v)	EC/TTE (w/w)
EC	10	0	0	100/0
EC/TTE0.5	9.1	0.5	8.82	53.1/46.9
EC/TTE1.0	8.3	1.0	17.6	36.2/63.8
EC/TTE1.5	7.7	1.5	26.4	27.4/72.6

EC: Ecovio® polymer blend; TTE: Tea tree essential oil.

Before electrospinning, the surface tension, electrical conductivity, and viscosity of the solutions were investigated. Surface tension was measured using a Lecomte Du Nouy K6 tensiometer (KRÜSS, Heidelberg, Germany) at 25°C, employing the Du Nouy method. The electrical conductivity of the solutions was estimated using a benchtop conductivity meter Mca-150, model MS Tecnopon at 25°C (São Paulo, Brazil). The apparent viscosity of the solutions was determined using a Ubbelohde viscometer (São Paulo, Brazil) at 25°C [33].

The EC and EC/TTE solutions were electrospun at a voltage of 12 kV, with a flow rate of 0.5 mL/h using an infusion pump (Harvard 2.2.2, Holliston, MA, USA). A copper plate covered with aluminum foil was used as a static collector. The solutions were electrospun whit a 10 mL syringe connected to a stainless-steel capillary needle (14 G; 2.1 × 40 mm) with a distance of 10 cm of the metallic collector. The produced fibers were labeled EC/TTEx, where x represents the volume of TTE added to the EC solution (Table 1).

2.3. Characterization

The morphology of the fibers was analyzed using Scanning Electron Microscopy (SEM) with a Thermo Fischer Scientific/Philips Quanta 250 apparatus (Prague, Czech Republic). The samples were coated with a gold layer of approximately 10 nm using cathodic spraying to enable electrical conduction. Contact angle measurements on the fiber surface were performed using the sessile drop method with Tantec A/S equipment (Lunderskov, Denmark). The fibers were characterized by Differential Scanning Calorimetry (DSC) using a DSC-60 Plus instrument (Kyoto, Japan) under an argon atmosphere (50 mL/h) at a heating rate of 10 °C/min over a temperature range of 25 to 300 °C. Infrared spectra in the attenuated total reflection mode (FTIR-ATR), ranging from 2000 to 650 cm^{-1},

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were obtained using a Shimadzu Scientific instrument, model 8300 (Kyoto, Japan), with an acquisition rate of 64 scans/min and a resolution of 4 cm^{-1} [37].

The chemical composition of the electrospun fiber surface was evaluated using X-ray Photoelectron Spectroscopy (XPS) with a Phi Electronics 5800 spectrometer (Chanhassen, MN, USA) equipped with a hemispherical analyzer and a multichannel detector. Spectra were obtained using a monochromatic Al Kα X-ray source (hν = 1486.6 eV). An energy analyzer with a pass energy of 23.5 eV and steps of 0.10 eV was used, with an X-ray spot size of 800 μm. All spectra were collected with a photoelectron take-off angle of 45°. Gaussian peaks were fitted according to the expected functional groups [38].

The mechanical properties of the fibers were evaluated according to ASTM D882-10 specifications. Measurements were performed using a MicroSystems texture analyzer (Surrey, England) on samples measuring 50 × 10 mm with an average thickness of 0.014 ± 0.001 mm (n = 10). The initial distance set on the instrument was 30 mm, with a crosshead speed of 0.083 mm/s, and 5 kg load cells were used [37].

2.4. Antimicrobial Assay with the Tea Tree Essential Oil and Fibers

The minimal inhibitory and bactericidal concentrations (MIC and MBC) of TTE oil were evaluated against *S. aureus* (ATCC® 25923) and *P. aeruginosa* (ATCC® 27853) bacteria using the microdilution method as reported by Balouiri et al. [39] with alterations. Suspensions (200 μL) of bacteria at 1.0 × 10^7 CFU/mL were placed in contact with the essential oil in a 48-well microdilution plate, containing 200 μL of Mueller-Hinton culture medium (pH 7.4 ± 0.2). The plate was then incubated for 24 hours at 37°C.

MIC is defined as the lowest concentration that inhibits bacterial growth. For MIC determination, after 24 hours of contact between the microbial suspension and TTE oil, 10 μL of a resazurin solution (0.1% w/v) was added to each well. The pink coloration of resazurin indicates high cellular activity and viability. Conversely, blue coloration of the dye indicates microbial growth inhibition. Thus, MIC is estimated based on a colorimetric test as reported elsewhere [40]. After 1 hour of contact with the resazurin dye, 10 μL of suspension from wells with blue coloration indicating microbial growth inhibition was collected. These aliquots were seeded onto Petri dishes containing Mueller-Hinton agar for 24 hours at 37°C to estimate the MBC, which is the lowest concentration that eliminates bacteria (99.99% or more) and prevents colony-forming units (CFUs) on Petri dishes. Control tests were also conducted. The positive control test involved adding 10 μL of microbial suspension (10^7 CFU/mL) without TTE oil presence to the Petri dish on Mueller-Hinton agar for 24 hours at 37°C. The negative test was performed by adding 10 μL of liquid culture medium in the presence of TTE oil but without bacteria on the Petri dish and Mueller-Hinton agar for 24 hours at 37°C.

The antimicrobial activity of EC/TTE fibers was assessed using the disk diffusion method on Muller-Hinton agar medium [41]. This method aimed to evaluate the diffusion capability of TTE within the microbial culture plate and the potential formation of inhibition zones against *S. aureus* (ATCC® 25923) and *P. aeruginosa* (ATCC® 27853) bacteria [42,43]. Before the antimicrobial assays with the Fibers (disks of 6 mm in diameter) were sterilized with ethylene oxide at 40°C for 120 minutes (G&S Sterilization of Health Products, Brazil) [34].

Bacteria were suspended in saline solution (0.9% w/v) and spread onto Muller-Hinton agar plates in Petri dishes (90×15 mm). Filter paper disks impregnated with TTE (10 μL) and EC/TTE fibers (6 mm diameter disks) were placed on the plates containing the bacteria. The plates were then incubated at 37°C for 24 hours. After incubation, the diameters (mm) of the inhibition zones formed around the disks were evaluated. A control assay was conducted using filter paper disks impregnated with TTE.

2.5. Anti-Adhesive Assay with Bacteria

The anti-adhesive properties of the fiber surfaces was evaluated according to Plath et al. [37] with alterations. Before the assay, fiber disks (6 mm in diameter) were attached to polystyrene disks

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(6 mm) using a double-sided tape. The prepared samples were sterilized with ethylene oxide at 40°C for 120 minutes (G&S Sterilization of Health Products, Brazil) [34].

The anti-adhesive properties of EC, EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 fiber surfaces were analyzed and compared with a negative control based on polystyrene disks (6 mm). Bacterial suspensions (500 µL) in Mueller-Hinton medium were inoculated onto the samples in a 48-well plate at 1.0×10^7 CFU/mL for 24 hours (n = 3). After incubation, the microbial suspension was removed, and the surfaces were washed once with PBS. The bacteria on the samples were fixed for SEM visualization using a solution containing 3% v/v glutaraldehyde (Sigma-Aldrich, São Paulo, Brazil), sodium cacodylate (0.1 M; Sigma-Aldrich, São Paulo, Brazil), and sucrose (0.1 M; Sigma-Aldrich, São Paulo, Brazil) for 45 minutes at room temperature. Subsequently, the samples were washed with 0.1 M sodium cacodylate and 0.1 M sucrose solution for 10 minutes each, followed by rinsing in water for 10 minutes. After removing the rinse water, the samples were freeze-dried for 24 hours, then gold-coated and imaged by SEM at an acceleration voltage of 10–20 kV.

2.6. Platelet Adhesion Assay

Whole blood was obtained from a healthy individual following a protocol approved by the Institutional Review Board of Colorado State University. Blood was collected in 10 mL EDTA-coated tubes (BD). Plasma, containing platelets and leukocytes, was separated by centrifugation at 100 × g for 15 minutes to remove red blood cells. Platelets and leukocytes were allowed to rest for 10 minutes before use. Plasma and cells were combined in a sterilized 50 mL conical tube. Sterilized samples (6 mm disks fixed on polystyrene disks) were placed in a 24-well plate and incubated with 200 µL of plasma for 2 hours at 37°C and 5% CO2 at 100 rpm. After incubation, the plasma was aspirated, and the samples were washed with PBS to remove non-adherent platelets from the fiber surfaces. Subsequently, a 2.0 µM calcein-AM solution in PBS was added to the samples and incubated for 30 minutes on an orbital shaker at room temperature. The solution was then aspirated, and the surfaces were washed with PBS three times. Platelet adhesion on the fiber surfaces was examined using Fluorescence Microscopy with a Zeiss Axiovision microscope (Dublin, USA). The assay was conducted in triplicate, with 10 fluorescence images captured in each replicate. Image analysis was performed using ImageJ Software to quantify the percentage of surface area covered by adhered platelets [44].

2.7. Whole Blood Coagulation Assay

To assess human blood clotting in the presence of fibers, sterilized samples (6 mm) were incubated with fresh blood (as-obtained from the donors) in a 24-well plate. A 7.0 µL drop of human blood was added to each fiber sample and left for 15 minutes. After incubation, the fibers were removed and transferred to another 24-well plate containing 500 µL of deionized water. The samples were agitated for 5 minutes on a horizontal shaker to release free hemoglobin and disperse non-coagulated blood cells. Subsequently, the absorbance of free hemoglobin was measured at 540 nm using a plate reader (FLUOstar Omega, BMG LABTECH, Cary, NC, USA).

2.7. Statistical Analysis

At least three different samples of each sample were used in all experiments; results are presented as mean ± standard deviation. Differences were determined using one-way ANOVA (p = 0.05) with a post-hoc Tukey's honest significant difference test.

3. Results and Discussion

3.1. Solution Properties

The TTE dissolves in the EC solutions prepared in a chloroform and formic acid mixture, leading to homogeneous solutions. The solution properties, including electrical conductivity, surface tension,

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and viscosity were investigated with the EC, EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 solutions (Table 1) and are compiled in Table 2.

Table 2. Solution properties (electrical conductivity, surface tension, and viscosity), average size of the electrospun fibers, and water contact angles (WCA) measured on the fibers.

Solutions	Conductivity (μS/cm)	Surface tension (mN/m)	Viscosity (mm²/s)	Size (nm) [1]	WCA (°) [1]
EC	1.12±0.12[a]	32.6±0.4[a]	9.8±0.2[a]	478±219[a]	120±2[a]
EC/TTE0.5	1.07±0.10[a]	28.1±0.2[b]	8.3±0.1[b]	393±120[b]	97±4[b]
EC/TTE1.0	0.63±0.08[b]	28.2±0.2[b]	8.2±0.2[b]	252±51[b]	95±5[b]
EC/TTE1.5	0.74±0.07[b]	30.1±0.2[c]	6.9±0.1[c]	278±59[b]	69±1[c]

[1] Results for average fiber size and water contact angles (WCA) were obtained from the electrospun fibers produced using the solutions listed in Table 1. Different superscript letters indicate statistically significant differences within each column ($p \leq 0.95$).

The addition of TTE to the solutions reduced the electrical conductivity from 1.12 μS/cm (EC solution) to 0.63 μS/cm in the EC/TTE solution with 1.0 mL of TTE. Similarly, surface tension decreased from 32.6 mN/m in the EC solution to about 28 mN/m in the EC/TTE solutions with 0.5 and 1.0 mL of TTE, and to 30.1 mN/m for the EC/TTE solution containing 1.5 mL of TTE (equivalent to 72.57% w/w of TTE relative to the mass of EC+TTE in the mixture). The viscosity of the EC solution (10% w/v), without TTE, was 9.6 mm²/s. In contrast, the EC/TTE solutions with 0.5 and 1.0 mL of TTE showed reduced viscosities of 8.3 and 8.2 mm²/s, respectively. The EC/TTE1.5 mixture exhibited a viscosity of 6.9 mm²/s.

These findings indicate that TTE oil significantly lowers viscosity, surface tension, and electrical conductivity of EC blends. Adjusting these parameters is crucial to optimize the electrospinning process. For example, Pavezi et al. [45] demonstrated that concentrations exceeding 10% v/v of acetic acid in chloroform/acetic acid binary mixtures can prevent the formation of microparticles during electrospraying of PLA solutions. By carefully controlling PLA concentration and solvent systems, they favored the electrospinning process over electrospraying. Additionally, Pavezi [45] emphasized that PLA (26 kDa) concentrations below 20% by mass do not result in fiber formation but rather in particle formation via electrospraying.

The presence of TTE in the EC solution decreased its surface tension, likely due to TTE containing amphiphilic compounds similar to surfactants, which are known to reduce surface tension in solutions [46]. The EC/TTE1.5 solution exhibited a slight increase in surface tension (30.1 mN/m) compared to the EC/TTE0.5 and EC/TTE1.0 solutions. This increase can be attributed to the higher concentration of TTE (1.5 mL), which corresponds to 72.57% (w/w) relative to the EC concentration in the final mixture. The higher amount of TTE likely compensates the surface tension reduction observed in lower TTE concentrations (about 28 mN/m in EC/TTE0.5 and EC/TTE1.0 solutions).

Viscosity measurements also reflected the influence of TTE concentration. The viscosity of the EC solution (without TTE) was 9.8 mm²/s. With the addition of 0.5 and 1.0 mL of TTE, the viscosity decreased to 8.3 and 8.2 mm²/s, respectively. This reduction in viscosity can be attributed to the dilution effect caused by the addition of TTE, which lowers the overall concentration of EC in the solutions (Table 1). The EC/TTE1.5 mixture exhibited a greater viscosity reduction (6.9 mm²/s), further supporting this trend.

Volumes of 0.5, 1.0, or 1.5 mL of TTE were added to a 10% w/v EC solution prepared in 5 mL of chloroform/formic acid (85/15 v/v). This addition reduced the concentration of formic acid in the final mixture, thereby decreasing the electrical conductivity of the solutions. Formic acid is polar and has a high dielectric constant (58.5) [45]. The conductivity reduction observed in EC/TTE solutions is associated with the dilution of formic acid, which decreases its ability to accumulate charges for a given potential difference.

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In summary, incorporating TTE into the EC solutions affects surface tension, viscosity, and electrical conductivity, with these properties varying depending on the concentration of TTE added to the polymer blend solution. These adjustments are critical for optimizing the electrospinning process of EC/TTE mixtures.

3.2. Fiber Characterization

EC solutions with and without TTE were electrospun under the experimental conditions detailed in Table 1. The EC/TTE solutions were prepared using a binary solvent mixture of chloroform and formic acid at a ratio of 85/15 v/v, with varying volumes of TTE added. Previous studies have established that EC fibers can be successfully spun from solutions with concentrations ranging between 10% and 12% w/v, using solvent systems such as chloroform/formic acid and dichloromethane/formic acid [33].

SEM images of the fibers are presented in Figure 1. Fibers without TTE exhibited a significant presence of beads and a heterogeneous structure, with an average diameter of 392 ± 131 nm. The incorporation of TTE led to a notable reduction in fiber diameter due to decreased surface tension and viscosity of the solutions. This effect facilitated greater elongation of the polymer blend jet during electrospinning, resulting in thinner and more uniform fibers. Specifically, the addition of 1.0 mL of TTE imparted the smallest average diameter (252 ± 51 nm) compared to other conditions. When 1.5 mL of TTE was used, there was a slight increase in average fiber diameter (278 ± 59 nm) compared to the 1.0 mL TTE condition. This increase may be attributed to the higher surface tension observed in the EC/TTE1.5 solution compared to EC/TTE1.0. However, the difference in average fiber diameter between EC/TTE1.0 and EC/TTE1.5 was not statistically significant (Table 2).

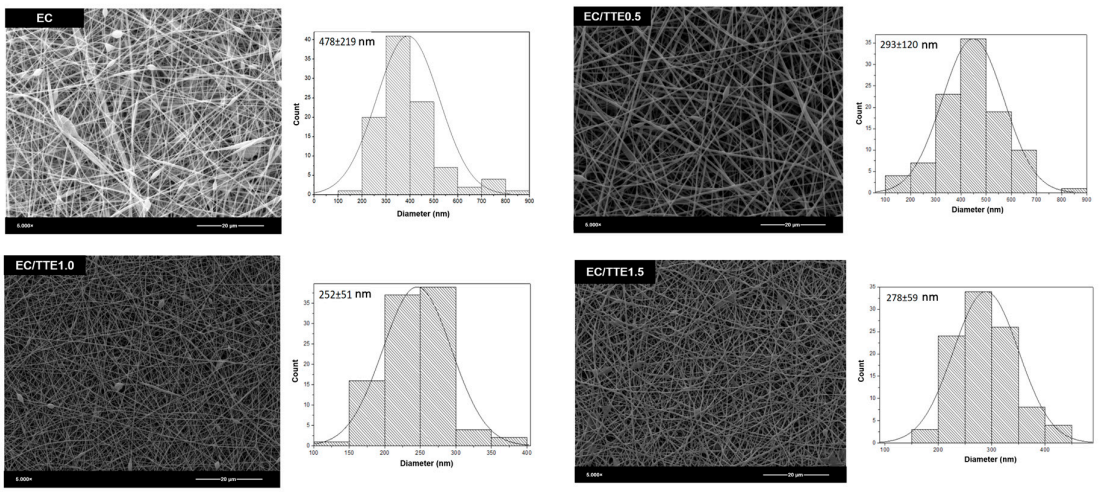

Figure 1. SEM images of EC and EC/TTE fibers obtained from the solutions listed in Table 1. Labels: EC = Ecovio® polymer blend composed of 55% poly(butylene adipate-co-terephthalate) (PBAT) and 45% poly(lactic acid) (PLA); TEE: Tea tree essential oil with a density of 0.8818 g/mL; EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 are the electrospun solutions which resulted in fibers after electrospinning, prepared by adding 0.5 mL, or 1.0 mL, or 1.5 mL of TTE into a EC solution at 10% w/v, respectively.

Using 0.5 mL of TTE resulted in fibers with an average diameter of 293 ± 120 nm, corresponding to an EC/TTE ratio of 53/47 w/w in the EC/TTE0.5 solution. Meanwhile, the EC/TTE1.0 solution with a ratio of 36/64 w/w generated fibers with an average diameter of 252 ± 51 nm, and the EC/TTE1.5 solution with a ratio of 27/73 w/w resulted in fibers with an average diameter of 278 ± 59 nm (Figure 1). Overall, the presence of TTE reduced the average fiber diameter and minimized bead formation, with the most pronounced effect observed in the EC/TTE1.0 and EC/TTE1.5 mixtures (Figure 1).

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An arithmetic mean of 150 random fiber diameter measurements was used for histogram representation in Figure 1. It is evident that the EC/TTE mixtures demonstrated optimal electrospinnability up to 1.5 mL of TTE added to the EC solution, beyond which there was a noticeable decrease in fiber homogeneity. The addition of TTE to the EC solutions resulted in a significant decrease in the average fiber diameter ($p \leq 0.95$) (Table 2). Furthermore, TTE enhanced the homogeneity of the fibers, leading to narrower size distribution curves (Figure 1).

The water contact angle reflects the adhesive and cohesive forces of a liquid on a surface. Surfaces are categorized as superhydrophilic when their water contact angles are below 40°, hydrophilic between 40° and 90°, hydrophobic between 90° and 120°, and superhydrophobic when angles exceed 120° [47,48].

Table 3 presents the water contact angle results (degrees) for the fibers: 120° ± 2 for the EC sample, 97° ± 4 for EC/TTE0.5, 95° ± 5 for EC/TTE1.0, and 69° ± 1 for EC/TTE1.5. The EC, EC/TTE0.5, and EC/TTE1.0 fibers exhibit hydrophobic characteristics, while the EC/TTE1.5 sample, with a contact angle of 69° ± 1, is characterized as hydrophilic. This change can be attributed to the presence of terpinen-4-ol alcohol, a major component of TTE. The alcohol increases the fiber's polarity, facilitating interaction with water molecules through intermolecular hydrogen bonding [47,48].

The presence of TTE significantly enhances the hydrophilicity of the fibers, with the most notable effect observed at 1.5 mL of TTE (69° ± 1). This volume reduces the contact angle from 120° ± 2 (for fibers containing only EC) to 69° ± 1 (for fibers obtained with 1.5 mL of TTE in the EC/TTE mixture).

Vidal et al. [49] emphasize the significance of hydrophilic and hydrophobic components in biomedical applications, particularly in wound treatment systems. They suggest that hydrophobic fibers, characterized by contact angles greater than 90°, are well-suited for use as wound dressings in treating skin wounds. The hydrophobic nature facilitates easy exchange and replacement of the material covering the wound. The EC, EC/TTE0.5 and EC/TTE1.0 fibers, with their low wettability and hydrophobic surfaces, meet the requirements for wound dressing applications when considering the wettability parameter alone.

Conversely, hydrophilic fibers, with contact angles less than 90°, are desirable for scaffolding applications where the material remains in contact with the tissue and aids in accelerating the healing process by promoting cell proliferation and tissue formation. In this context, the EC/TTE1.5 sample, exhibiting increased wettability, shows potential for application as a scaffold material. However, additional properties such as surface roughness, mechanical strength, liquid absorption capacity (swelling behavior), biodegradability, stability, antimicrobial activity, and hemocompatibility, must be thoroughly investigated to determine the most suitable application for each material.

Figure 2 presents the DSC curves of EC and EC/TTE fibers. The EC DSC fiber profile displays an intense endothermic peak at 153°C, which is more pronounced than the endothermic peaks observed in the DSC curves of the EC/TTE fibers. Additionally, increasing the TTE volume from 0.5 to 1.5 mL shifts the endothermic peak to lower temperatures compared to the endothermic peak temperature in the EC fiber DSC curve. The endothermic peak in the EC/TTE1.0 fiber DSC curve occurs at 153°C, while in the EC/TTE1.5 sample, it shifts to 146°C.

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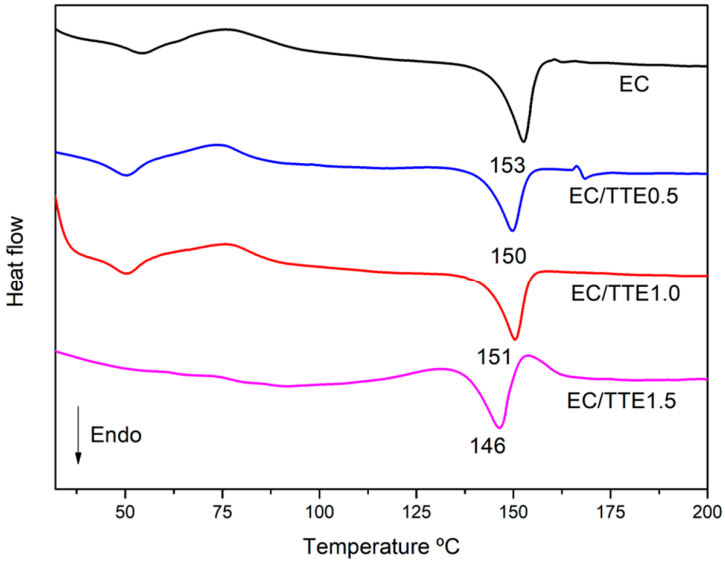

Figure 2. DSC curves of EC and EC/ETT fibers. Labels: EC = Ecovio® polymer blend composed of 55% poly(butylene adipate-co-terephthalate) (PBAT) and 45% poly(lactic acid) (PLA); TEE: Tea tree essential oil with a density of 0.8818 g/mL; EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 are the electrospun solutions which resulted in fibers after electrospinning, prepared by adding 0.5 mL, or 1.0 mL, or 1.5 mL of TTE into a EC solution at 10% w/v, respectively.

The presence of TTE is expected to alter the configuration of polymer chains in the blend, influencing interactions (especially hydrophobic interactions) between polymer species in the EC blend. This effect modifies the profile of the endothermic peak in the DSC curves, altering the melting temperature of the EC blend in the presence of TTE. This change in the melting profile helps to explain the formation of thin fibers when TTE is present, as demonstrated in the SEM images.

Alves et al. [50] presented DSC curves of poly(3-hydroxybutyrate)/poly(ethylene glycol) films incorporated with rosemary essential oil. The presence of the essential oil shifted the endothermic peaks related to the polymeric species to lower temperatures compared to the endothermic peak in the films obtained without the essential oil. Increasing the dosage of essential oil in the polymeric blend resulted in a reduction in the material's final melting temperature. The results presented in this study align with those of Alves et al. [50], suggesting that the essential oil impacts both the surface and bulk properties of the fibers. This alters the wettability and the profile of the DSC curves, respectively.

The FTIR-ATR spectra of TTE and the EC and EC/TTE1.5 fibers are presented in Figure 3. Intense and characteristic bands in the TTE FTIR spectrum at 1465, 1443, and 1378 cm^{-1} are attributed to the stretching of C-H bonds of methylene groups ($–CH_2$) [51]. The band at 872 cm^{-1} is attributed to the stretching of C-O-C, which is present in the compound 1,8-cineole [51].

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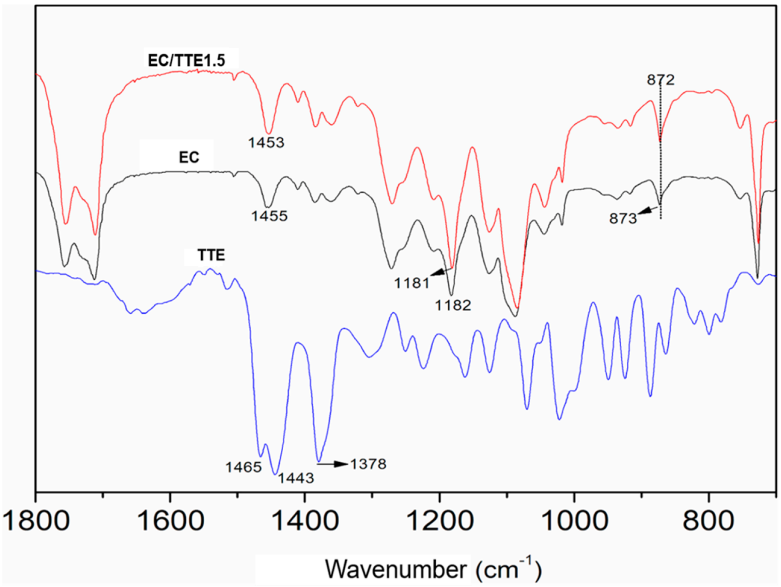

Figure 3. FTIR-ATR spectra of TTE, EC Fiber, and EC/TTE1.5 Fiber.

The FTIR spectra of the EC and EC/TTE1.5 fibers show bands at 1453 and 1455 cm^{-1}, attributed to the stretching of the C=O bond (carboxylic acid and ester portions) found in Ecoflex® and PLA, respectively [33]. The bands at 1453 and 1455 cm^{-1} are also attributed to the asymmetric and symmetric stretching of C-H bonds in Ecoflex® and PLA [33]. The bands at 1181 and 1182 cm^{-1} correspond to the symmetric and asymmetric stretching of C–O and C–O–C bonds (ester and carboxylic acid) found in Ecoflex® and PLA [34]. These bands confirm the presence of EC in the fibers. The band at 1181 cm^{-1} in the FTIR spectrum of the EC fiber shifts to 1182 cm^{-1}. A similar effect occurs with the band at 1455 cm^{-1} in the FTIR spectrum of the EC fiber, which shifts to 1453 cm^{-1} and increases in intensity due to the presence of TTE in the fiber. This result indicates the presence of TTE in the EC fibers.

Figure 4 shows the high-resolution XPS spectra of the carbon envelopes (C1s) obtained on the surface of EC and EC/TTE1.5 fibers. The C1s envelopes exhibit peaks corresponding to aliphatic carbon atoms (C-C at approximately 286 eV), carbon atoms bonded to oxygen (C-O near 288 eV), and carbon atoms in carbonyl groups (C=O at approximately 290 eV). The chemical composition of TTE primarily includes hydrocarbons, terpenes, and alkyl alcohols [52]. Consequently, TTE is rich in aliphatic carbon. This composition explains the increase in the relative percentage of aliphatic carbon from 48.51% on the surface of EC fibers to 57.55% on the surface of EC/TTE1.5 fibers (Figure 4). The increase in aliphatic carbon content results in a corresponding decrease in the relative percentage of oxygen, which accounts for the reduction in C-O groups from 25.94% (EC fiber) to 22.94% (EC/TTE1.5 fiber) and the reduction in C=O groups from 25.55% (EC fiber) to 20.03% (EC/TTE1.5 fiber). These changes confirm the incorporation of TTE into the EC/TTE fibers.

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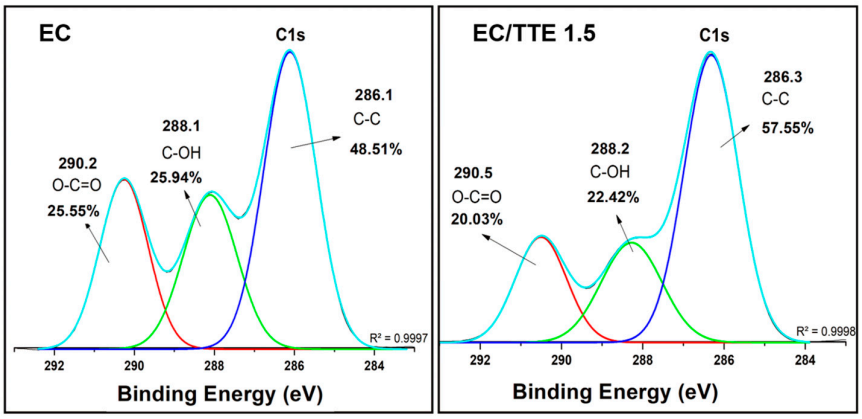

Figure 4. High-Resolution XPS Spectra for C1s Peaks obtained from EC and EC/TTE1.5 Fibers.

Figure 5 displays the stress-strain curves of the electrospun fibers. The mechanical properties of the EC fibers (478 nm) included a tensile strength of 0.00254 MPa, elongation at break of 6.61%, and a Young's modulus of 0.061 MPa. For the fibers containing TTE, the mechanical properties were as follows: EC/TTE0.5 (293 nm) had a tensile strength of 0.00654 MPa, elongation at break of 3.70%, and Young's modulus of 0.240 MPa; EC/TTE1.0 (252 nm) had a tensile strength of 0.00497 MPa, elongation at break of 4.91%, and Young's modulus of 0.111 MPa; and EC/TTE1.5 (278 nm) had a tensile strength of 0.00707 MPa, elongation at break of 4.40%, and Young's modulus of 0.283 MPa.

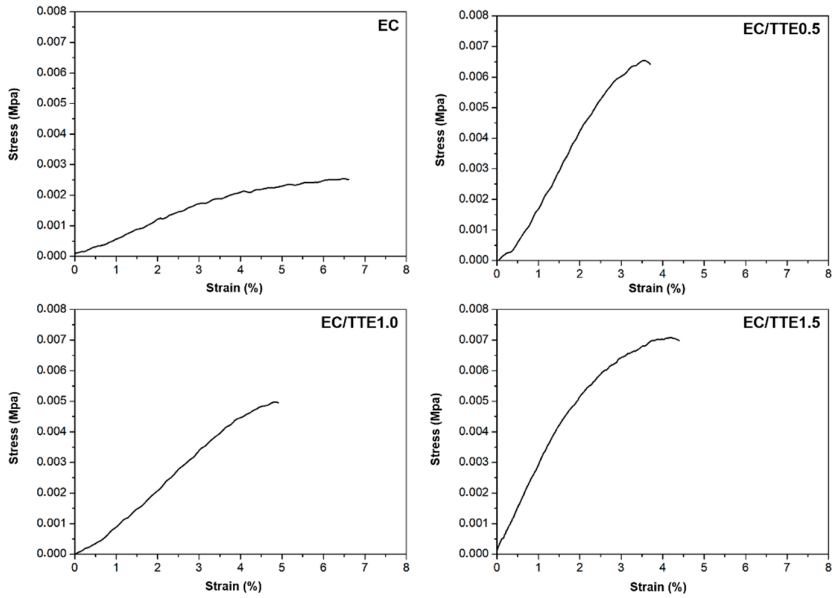

Figure 5. Stress-strain curves of the fibers.

The incorporation of TTE into the fibers significantly enhanced their mechanical properties. The presence of TTE increased both the tensile strength and the Young's modulus, indicating that TTE promotes the formation of stronger fibers with reduced plasticity. Specifically, TTE exhibited an anti-plasticizing effect, as evidenced by the substantial increase in the Young's modulus for EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 fibers compared to the EC fibers.

3.2. Antimicrobial Activity of the Tea Tree Essential Oil

Table 3 presents the MIC and MBC results of TTE against P. aeruginosa and S. aureus. High concentrations (about 7.5 mg/mL) of TTE are required to inhibit and achieve bactericidal activity.

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Other studies have indicated MIC and MBC in the range of 17 to 35 mg/mL against 30 different isolates of S. aureus [53]. These MIC and MBC results were estimated from percentage volume/volume concentrations, considering a density of 0.8819 g/mL for TTE. No MIC and MBC data for P. aeruginosa have been reported in the literature. However, the major component of TTE, terpinen-4-ol, has shown MIC and MBC in the range of 8% v/v against P. aeruginosa [54]. When this value is converted to mg/mL using the density of 0.8819 g/mL for TTE, the MIC and MBC values are approximately 70 mg/mL. The results obtained in this study differ slightly from those reported in the literature, especially concerning S. aureus. These differences are likely due to the variable composition of TTE.

Table 3. MIC and MBC of TTE against P. aeruginosa (ATCC® 27853) and S. aureus (ATCC® 25923).

Bacteria	MIC/MBC (mg/mL)
P. aeruginosa	7.5/7.5
S. *aureus*	7.5/7.5

Figure 6 shows digital images of Petri dishes containing agar seeded with microbial suspensions after MIC determination. The upper panel illustrates the effect of TTE concentration on *S. aureus*. In the *S. aureus* test, microbial growth inhibition at concentrations of 7.5 mg/mL and 15 mg/mL reached percentages higher than 99.999%. This result is based on the initial concentration of *S. aureus* (500 µL at 10^7 CFU) seeded with fibers in the MIC determination microdilution test. At a concentration of 3.75 mg/mL, there was no inhibition of *S. aureus*. Increasing the dosage to 7.5 mg/mL resulted in a reduction of colony-forming units (CFU) to 12. At a concentration of 15 mg/mL, the number of colonies reduced to just 3, indicating inhibition higher than 99.9999%.

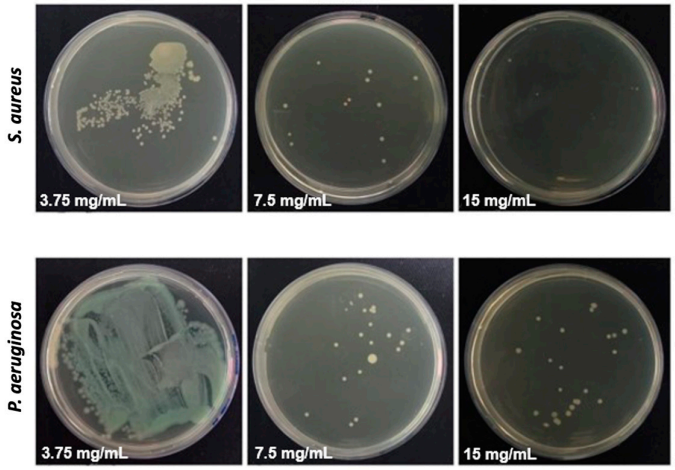

Figure 6. Digital images of Petri dishes containing agar seeded with 10 µL of microbial suspension of *S. aureus* and *P. aeruginosa* after MIC determination.

The lower panel presents the results against *P. aeruginosa* (Figure 6). A concentration of 3.75 mg/mL of TTE did not inhibit microbial growth; however, concentrations of 7.5 mg/mL and 15 mg/mL significantly reduced the CFU to 20 and 19 (about 99.999% of inhibition), respectively. These results confirm the antimicrobial activity of TTE against both gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*P. aeruginosa*).

3.3. Antiadhesive and Antimicrobial Activity of the Fibers

SEM images of polystyrene control and fibers after 24 hours of contact with S. aureus and P. aeruginosa are presented in Figure 7. Generally, bacteria adhered more to the surface of the polystyrene control and EC fibers. A greater number of S. aureus cells are observed on the fibers

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compared to P. aeruginosa cells. The S. aureus adhered to the surface of the polystyrene control and fibers exhibit regular morphology (cocci, spheres), indicating that the samples do not have cytotoxic effects on gram-positive S. aureus cells [55–58].

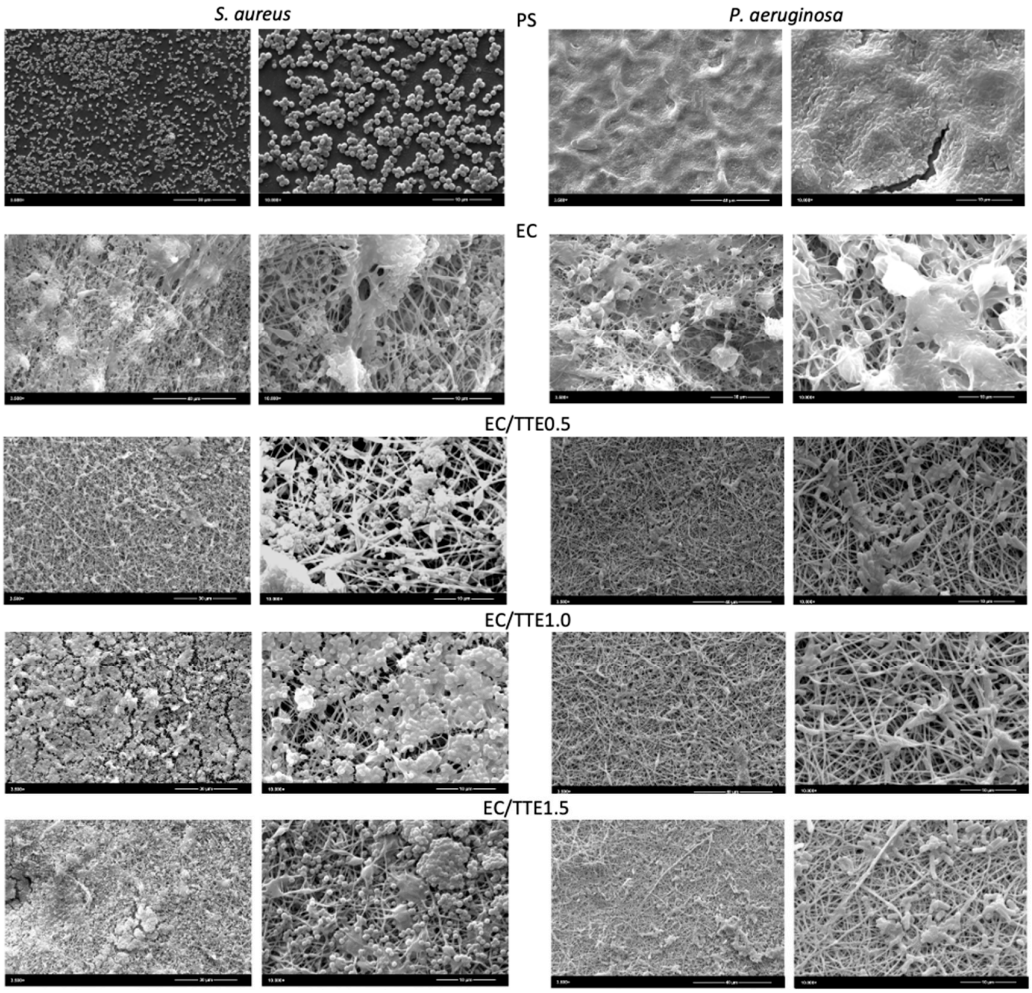

Figure 7. SEM images of polystyrene control (named as PS) and EC/TTE fibers seeded with S. aureus and P. aeruginosa after 24 hours of contact.

In contrast, P. aeruginosa cells did not completely cover the surfaces of EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 fibers as they did with the polystyrene control and EC fibers. This suggests that the fiber surface exhibits antiadhesive potential against P. aeruginosa, confirmed by control tests with the EC fiber and polystyrene film. P. aeruginosa appears to adhere more to the surface of TTE-free fibers than to fibers containing TTE. Adhesion is also more pronounced on the polystyrene surface, as expected, since this material lacks antiadhesive and antimicrobial activity [12].

EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 fibers seems to demonstrate biocidal activity against P. aeruginosa. Some microbial cells adhered to the surface of TTE-containing fibers do not exhibit the regular rod-shaped morphology (bacilli) seen on the surface of polystyrene. The presence of irregularities and greater heterogeneity in EC fibers complicates the comparison of microbial adhesion and proliferation results with TTE-containing fibers. However, P. aeruginosa cells with regular morphology are also observed on the surface of EC fibers. Additionally, regions where P. aeruginosa cells are aggregated on the surface of EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 fibers are visible, particularly in EC/TTE1.5 fiber.

The disk diffusion assay did not indicate the formation of inhibition zones against S. aureus and P. aeruginosa (not shown). It is suggested that the TTE interacts through attractive van der Waals

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forces with the fibers, as mechanical tests indicated an anti-plasticizing effect. These interactions (dipole-dipole and London dispersion) likely inhibit the release of TTE onto the agar-seeded Petri dish, thereby preventing the formation of inhibition zones.

3.4. Blood Coagulation

When any injury exposes blood, the body initiates the coagulation process, involving physical and chemical changes in the blood through various factors. Platelet adhesion on fiber surfaces indicates thrombogenicity, which can lead to platelet activation and the start of the coagulation cascade [49]. During this process, blood loses its fluid properties and solidifies due to coagulation. Wound dressings should facilitate blood coagulation in damaged skin tissues to prevent bleeding.

Figure 8 shows fluorescence images of fibers and a control (glass surface) in contact with human blood platelets, stained with calcein-AM dye, after 2 hours of incubation. Calcein-AM highlights the platelets adhered to the fiber surface, appearing as green staining. Platelet adhesion on the fiber surface indicates that blood coagulation is likely to occur on the fiber surface, suggesting low hemocompatibility [49].

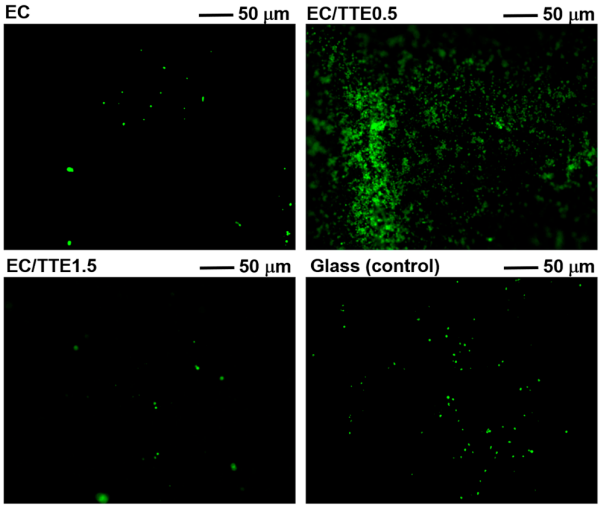

Figure 8. Fluorescence images of fibers and control test (glass) after contact with human blood platelets for 2 hours of incubation. Platelets were stained with calcein-AM, emitting green coloration in the fluorescence images.

Fluorescence images reveal that platelets preferentially adhere to the surface of EC/TTE0.5 fiber, even compared to the control assay conducted with glass, which is known to be non-hemocompatible. This result implies that the surface of EC/TTE0.5 fiber is prone to blood coagulation, indicating low blood compatibility. Overall, platelets adhered to all fiber surfaces, as confirmed by the fluorescence images (Figure 8).

The percentage of surface area covered by platelets is presented in Figure 9. The area covered by platelets on the surface of EC/TTE0.5 fiber was 17.5%, which is significantly higher than the coverage on other samples, none of which exceeded 0.20% ($p \leq 0.05$). This result suggests that EC/TTE0.5 fiber may be suitable for covering damaged skin tissues exposed to bleeding. In such cases, the fiber could act as a wound dressing, promoting blood coagulation on the skin and preventing blood loss at the injury site.

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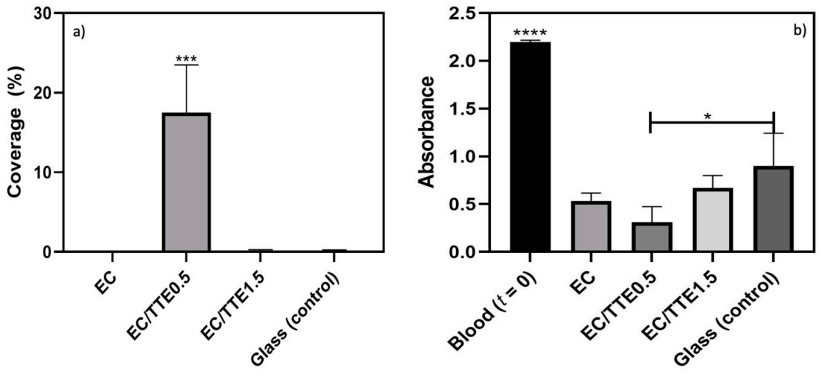

Figure 9. (a) Percentage of surface area covered by platelets obtained through analysis of fluorescence images using ImageJ software. The **** indicates significant results with $p \leq 0.001$. (b) Blood coagulation results of human blood exposed to the fibers and control (glass). The term "blood (t = 0)" in Figure "b" refers to the absorbance of hemolyzed erythrocytes obtained from fresh human blood at time zero, meaning before any contact with the fibers and glass.

The fluorescence images indicate platelet deposition on the surface of all samples, though it is less pronounced in the EC and EC/TTE1.5 samples. This suggests a low compatibility between human blood and the fibers. Figure 10b confirms these findings through UV-Vis absorbance measurements taken after bringing the fibers and a glass control sample into contact with human blood for 15 minutes. Coagulation reduces the concentration of suspended red blood cells (erythrocytes), thus decreasing absorbance at 540 nm, as coagulated blood loses its reddish coloration.

At t=0 , the absorbance of human blood was around 2.195. After 15 minutes, the absorbance values dropped to 0.531 for the blood in contact with EC, 0.309 for EC/TTE0.5, and 0.668 for EC/TTE1.5 (Figure 10b). These results indicate that all surfaces induce coagulation, but the EC/TTE0.5 fiber exhibits a significant effect. Notably, the EC/TTE0.5 sample was the only one that showed statistically different coagulation results compared to the glass control sample ($p \leq 0.05$), which supported an absorbance of 0.901 after 15 minutes of contact (Figure 10b).

4. Conclusions

This research aimed to produce electrospun fibers with enhanced biological properties in vitro aiming suitable for creating wound dressings to protect skin lesions and prevent the growth and proliferation of bacteria. The electrospinning technique used to fabricate Ecovio® (EC) fibers encountered challenges due to the hydrophobic nature and solubility of EC in low polarity solvents. To address this, tea tree essential oil (TTE) was added to EC solutions at volumes of 0, 0.5, 1.0, and 1.5 mL, resulting in EC, EC/TTE0.5, EC/TTE1.0, and EC/TTE1.5 fibers, respectively. Without TTE, it was not possible to produce fine and homogeneous EC fibers free of beads. The addition of TTE improved fiber homogeneity and enabled the production of thin and bead-free fibers, with an average diameter of 252±51 nm.

TTE not only enhanced fiber homogeneity but also altered their wettability, with 1.5 mL of TTE producing hydrophilic fibers. Additionally, TTE affected the DSC curve profiles, shifting endothermic peaks to lower temperatures and reducing their intensities. TTE also modified the mechanical properties of the fibers, increasing their elasticity modulus. The antimicrobial results demonstrated that TTE provided significant activity against *S. aureus* and *P. aeruginosa* at 7.5 mg/mL, inhibiting at least 99.99% of bacteria in suspension. EC/TTE fibers exhibited antiadhesive and cytotoxic properties against *P. aeruginosa*, with reduced bacterial growth on the fiber surfaces. However, EC/TTE fibers, particularly those with 0.5 mL of TTE, showed low hemocompatibility even as compared with glass. In conclusion, EC/TTE fibers possess several properties desirable for biomedical wound dressings, including inhibition of bacterial adhesion and growth, and the ability

to coagulate human blood. Therefore, EC/TTE0.5 and EC/TTE1.5 fibers may have potential applications in treating skin wounds.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization, B.Z.d.S., D.P.F., and A.F.M.; methodology, B.Z.d.S., D.P.F., and S.P.F.; software, B.Z.d.S. and A.F.M.; validation, B.Z.d.S. and E.G.B.; formal analysis, C.F.T., D.A.d.A., and A.F.M.; investigation, B.Z.d.S., D.P.F., S.P.F., and D.A.d.A.; resources, M.J.K., K.C.P., and A.F.M.; writing—original draft preparation, B.Z.d.S. and C.F.T.; writing—review and editing, E.G.B., M.J.K., K.C.P., and A.F.M.; visualization, A.F.M.; supervision, A.F.M. and E.G.B.; project administration, A.F.M., and E.G.B. All authors have read and agreed to the published version of the manuscript.

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