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Activity of Wet-Spun Fibers Chemically Modified with Active Biomolecules Against Gram-Positive and Gram-Negative Bacteria †

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Abstract: Essential oils (EOs), which are complex biomolecules composed of volatile compounds, have emerged as a new strategy to deal with bacterial infections and as a valid alternative to synthetic drugs. Here, we report the production and modification of wet-spun microfibers made of cellulose acetate (CA) and polycaprolactone (PCL) with the EOs cinnamon leaf oil (CLO), cajeput oil (CJO), and clove oil (CO). These were selected from a group of 20 EOs according to their minimal inhibitory concentration (MIC) against *Staphylococcus aureus* (<22.4 mg/mL) and *Escherichia coli* (<11.2 mg/mL) bacteria. Microfibers were produced by wet-spinning at an extrusion rate of 0.5 mL/h directly into an ethanol coagulation bath. EOs loading was accomplished by immersion in ethanol solutions containing the EOs at 2xMIC. Incorporation was confirmed by UV-Visible spectroscopy and Fourier-transformed infrared spectroscopy. After 72 h of incubation, microfibers contained 14%, 66% and 76% of the MIC values of CLO, CO and CJO, respectively. Unloaded and loaded microfibers were characterized as uniform and homogeneous; no significant differences were detected. EO-modified microfibers were effective against the tested bacteria. Considering the amount immobilized, CLO-containing fibers were deemed the most effective from the group, suggesting a superior affinity of the EOs active groups towards the CA/PCL matrix. These results indicate that CA/PCL microfibers loaded with EOs have potential for biomedical application in which infection control is the target.

Keywords: microfibers; biocompatible polymers; essential oils; antibiotics; surface modification; bactericidal effect; localized biomolecule action

1. Introduction

Antimicrobial resistance in bacterial pathogens is a worldwide concern that is being progressively associated with increasing morbidity and mortality rates. Despite repeated warnings, negligent antibiotic use and poor infection-control practices have aggravated this issue turning it into a global crisis [1]. Natural alternatives are being proposed to replace the overused drugs. Plant extracts, for instance, have long been applied in traditional medicine and are now gaining renewed attention for their inherent antimicrobial an anti-inflammatory potential. Indeed, they are now being associated with fibrous-based, localized delivery platforms that are capable of enhancing their topical action, this way improving their overall effectiveness [2,3]. Essential oils (EOs) are between those

selected natural compounds with an antimicrobial action of interest. Indeed, these aromatic, lipophilic biomolecules, work as secondary metabolites within plants exerting functions of defense against microbial invasion [4]. EOs display a broad spectrum of antimicrobial activity against bacteria, fungi, and viruses, and strong anti-inflammatory, antiseptic, analgesic, spasmolytic, anesthetic, and antioxidative properties [5]. Due to their volatile nature, fibers and fibrous constructs have been researched as delivery platforms for these biomolecules [3].

The wet-spinning technique is based on the principle of precipitation in which fiber production occurs as the contact of a polymer solution with a coagulation, containing a non-solvent bath, is made [6]. This technique allows the production of 3D intricated fibrous constructs that promote cell infiltration, something that is very difficult to attain in electrospun nanofibrous systems. Further, it can be applied to both natural and synthetic polymers and easily loaded with a wide range of therapeutic agents. In the present study, blends of cellulose acetate (CA) and polycaprolactone (PCL) were processed by wet-spinning and loaded with selected EOs with the purpose of being explored as drug-delivery platforms applied to the combat of bacterial infections.

2. Materials and Methods

Fiber production and examinations were divided in seven steps. The overall goal is highlighted in Figure 1.

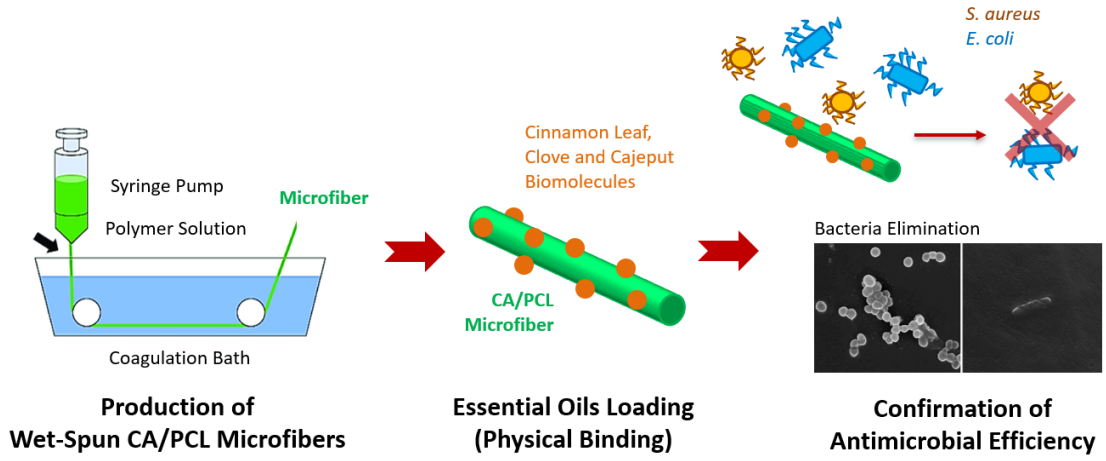


Figure 1. Schematic representation of the production of microfibers from blends of CA and PCL loaded with EOs and their antimicrobial testing against *S. aureus* and *E. coli* bacteria [7].

2.1. Polymeric Solution Preparation

Polymers—cellulose acetate (CA, Mn = 30,000 and 39.8 wt.% acetyl content) and polycaprolactone (PCL, Mn = 45,000).

Solvents—acetone/acetic acid at 3:7 *v*/*v*.

Polymer ratio—3:1 CA/PCL (10/14 wt.%).

Solubilization conditions—1 h at 75 °C and 200 rpm.

2.2. Wet-Spinning Processing Conditions

Flow rate—0.5 mL/h.

Needle Gauge—18.

Coagulation bath—Ethanol.

Temperature of extrusion—21 to 22 °C.

2.3. Minimum Inhibitory Concentration Studies

EOs—Amyris, Cajeput (CJO), Cinnamon leaf (CLO), Citronella, Clove (CO), Eucalyptus, Frankincense, Geranium, Himalayan cedar, Lavandin, Lemongrass, Niaouli, Orchid, Palmarosa, Patchouli, Rosemary, Sage, Star anise, Tea tree oil, and Wintergreen.

Bacteria—Staphylococcus aureus (S. aureus, ATCC 6538) and Escherichia coli (*E. coli*, ATCC 25922).

Concentration—1×10^7 CFUs/mL in Muller Hinton Broth.

Incubation period—24 h.

2.4. Loading Efficiency

EOs concentration—2× MIC value in ethanol.

Conditions—72 h at room temperature and 200 rpm, protected from light.

Confirmation—UV-visible spectroscopy at 280 nm.

2.5. Chemical Characterization

Equipment—Attenuated Total Reflectance with Fourier-Transform Infrared Spectroscopy (ATR-FTIR), IRAffinity-1S, SHIMADZU spectrophotometer (Kyoto, Japan).

Crystal—diamond.

Conditions—45 scans, with spectral resolution of 8 cm^{-1}, from 400–4000 cm^{-1}.

2.6. Antimicrobial Testing

Evaluation—time kill kinetics.

Incubation periods—1, 2, 6 and 24 h at 37 °C, under 120 rpm.

Bacteria—S. aureus and *E. coli*.

Concentration—1×10^5 CFUs/mL in Tryptic Soy Broth.

2.7. Membrane Permeabilization

Examinations—differences in relative electric conductivities (REC).

Bacteria—S. aureus and *E. coli*.

Concentration—1×10^5 CFUs/mL in Muller Hinton Broth.

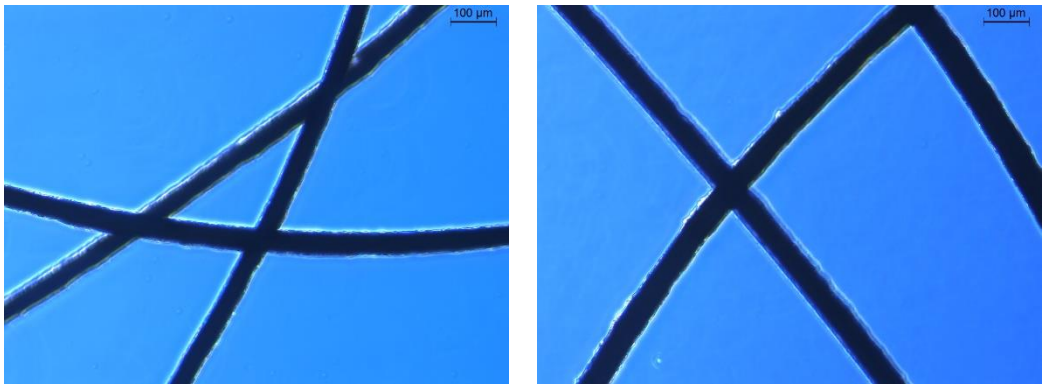
3. Results and Discussion

EOs were examined individually for their antimicrobial action against the Gram-positive bacteria *S. aureus* and the Gram-negative bacteria *E. coli*. CLO, CO and CJO were considered the most effective from a group of 20 EOs, with MICs values of 0.82, 0.83 and 22.38 mg/mL against *S. aureus* and 0.82, 0.83 and 11.19 mg/mL against *E. coli*, respectively.

Microfibers of CA/PCL were successfully wet-spun from a spinning solution prepared at a 3:1 *v*/*v* polymer ratio. The fibers presented a homogeneous, continuous, and uniform appearance, with no detectable defects (Figure 2). Average diameters ranged between 54 and 59 μm regardless of the presence or absence of loaded EOs. There were no significant differences introduced by any of the specific loaded EOs. Loading occurred by physical adsorption via the immersion of the microfibers, for a 72 h period, in an ethanol-based solution containing CLO, CO and CJO at 2× MIC. Fiber loading reached only 14%, 66%, and 76% of the MICs of CLO, CO and CJO respectively. Regardless of their amount, ATR-FTIR peaks characteristic of the EOs were identified on the fibers, namely at 1577 and 1543 cm^{-1}, which represented the aromatic ring C=C skeleton vibration of an aromatic substance, and at 1452 cm^{-1}, which was attributed to the –C–OH bending vibration from the EOs alcohol moieties [8–11].

The antimicrobial action of the free oils and the loaded fibers was followed by mapping the time kill kinetics of the fibers from 1 h to 24 h of incubation. Data reported bacteria reduction from the first moments of interaction, with the free EOs being more effective than the loaded fibers, as expected.

After 24 h culture, it was evident that *S. aureus* was more susceptible to the prolonged action of the EOs than the *E. coli*. The only exception was the action of CJO, since this oil presented a smaller MIC for *E. coli* and was loaded in a higher amount than CLO or CO. The mechanisms of action of the EOs against the bacteria, namely membrane permeation, were also confirmed via permeabilization studies using the loaded microfibers, in which, once again, the *S. aureus* was the most susceptible to the action of the oils [7,12].



(**a**) (**b**)

Figure 2. Example of the obtained fibers morphology before (**a**) and after (**b**) EOs loading.

4. Conclusions

The versatility of the wet-spinning technique is one of its most important features. Its ability to offer new opportunities for the incorporation of various antimicrobial agents remains of great importance to biomedical applications. This research is an example of that. Indeed, the results demonstrated the potential of CA/PCL wet-spun microfibers loaded with EOs for applications in biomedicine, in which treatment of infections caused by the Gram-positive bacteria *S. aureus* and the Gram-negative bacteria *E. coli* are a main target.

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Conflicts of Interest: The authors declare no conflict of interest.

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