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Bactericidal action of cinnamon, clove and cajeput oils loaded onto CA/PCL wet-spun fibers for a localized, controlled biomolecule delivery

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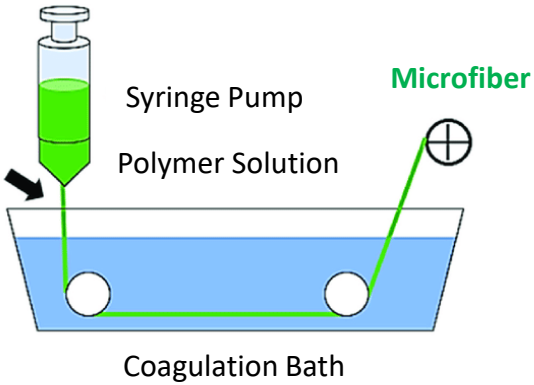
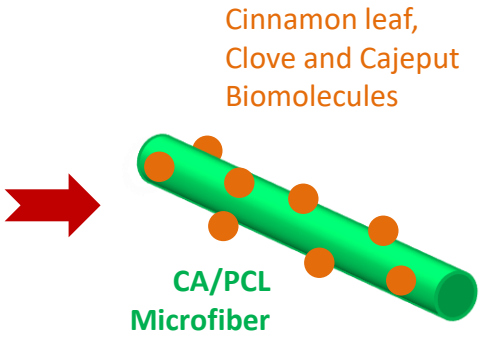
CENTRO DE CIÊNCIA E TECNOLOGIA TÊXTIL

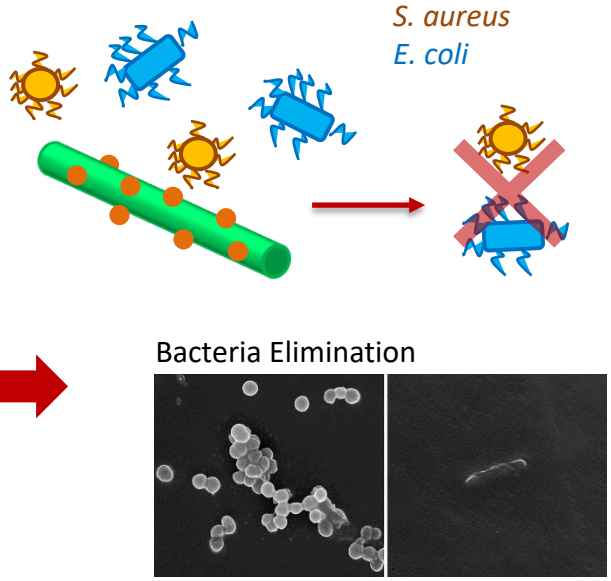
Bactericidal action of cinnamon, clove and cajeput oils loaded onto CA/PCL wet-spun fibers for a localized, controlled biomolecule delivery



Production of Wet-Spun CA/PCL Microfibers



Essential Oils Loading (Physical Binding)



Confirmation of Antimicrobial Efficiency

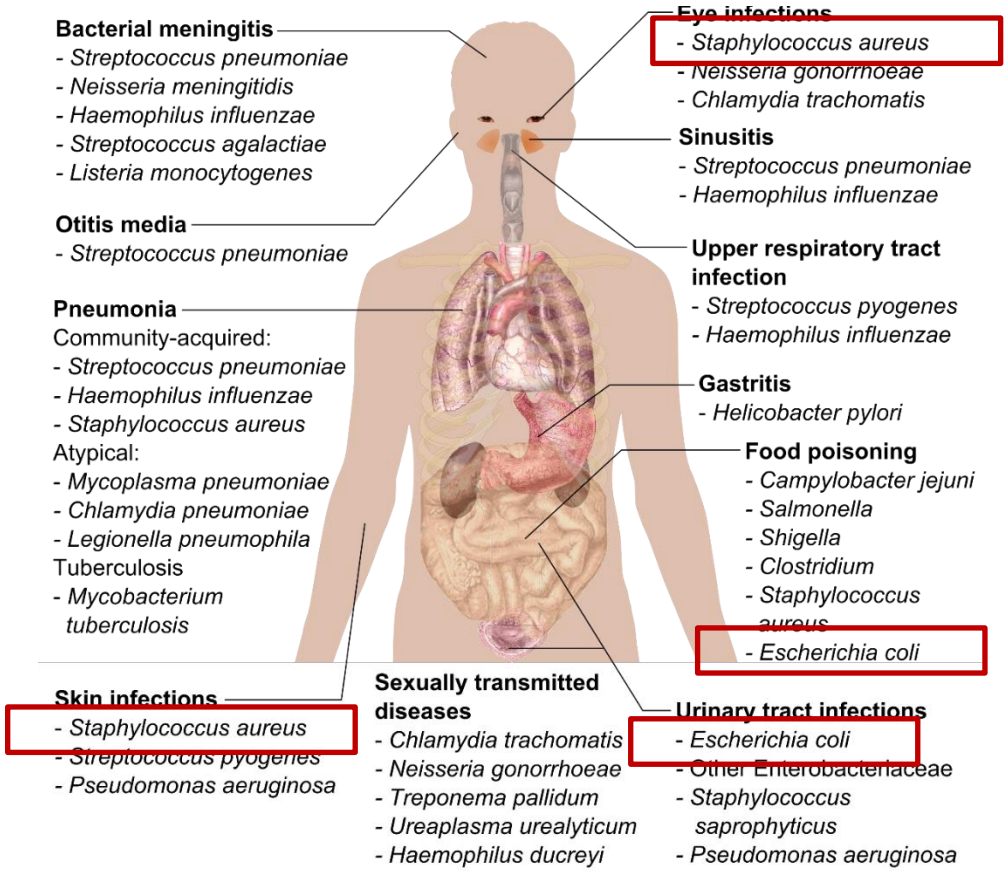
(Representation not to scale)

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 modification of biodegradable wet-spun microfibers composed of cellulose acetate (CA) and polycaprolactone (PCL) with EOs, aiming at their localized, controlled release. Cinnamon leaf oil (CLO), cajeput oil (CJO), and clove oil (CO) 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). CA/PCL prepared at 10% and 14%wt in a 3/1 ratio in acetic acid and acetone were processed in the form of microfibers by wet-spinning at an extrusion rate of 0.5 mL/h directly into an ethanol coagulation bath. Microfibers were modified by immersion in ethanol solutions containing EOs at 2xMIC and ampicillin (control antibiotic). Incorporation was confirmed by UV-VIS, FTIR and TGA. After 72h, fibers contained ampicillin at MIC but only 14%, 66% and 76% of MIC for CLO, CO and CJO, respectively. Unloaded and loaded microfibers were characterized as uniform and homogeneous. Data showed that even at small amounts the 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 can be easily produced and applied in scaffolds for biomedical applications.

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

Introduction - Bacterial Infections



Recent projections indicate that bacterial infections may be the cause of approximately **10 million annual deaths worldwide by 2050**

Willyard, C.. *Nature* **2017**, *543*, 15.

Introduction - Conventional Treatments vs Essential Oils (EOs)

Antibiotics/Antiseptic Agents

Target bacterial functions, growth processes or the bacterial cell wall - **bactericidal** activities.

⇩

Rising of antibiotic resistant pathogens

⇩

Inefficient

Essential Oils

EOs are produced by more than **17,500 species** of plants

⇩

Volatile biomolecules endowed with **antimicrobial and regenerative potential**

⇩

Alternative

Tavares et al., Antibiotics 2020, *9*, 314 - doi:10.3390/antibiotics9060314; Tavares et al., Biomolecules 2020, 10, 148 - doi:10.3390/biom10010148

Introduction - EOs Drawbacks

- cytotoxic at increased concentrations, which prevents systemic delivery;
- present low resistance to degradation by external factors (e.g. temperature, light, moisture);
- highly volatile in their free, unloaded form.

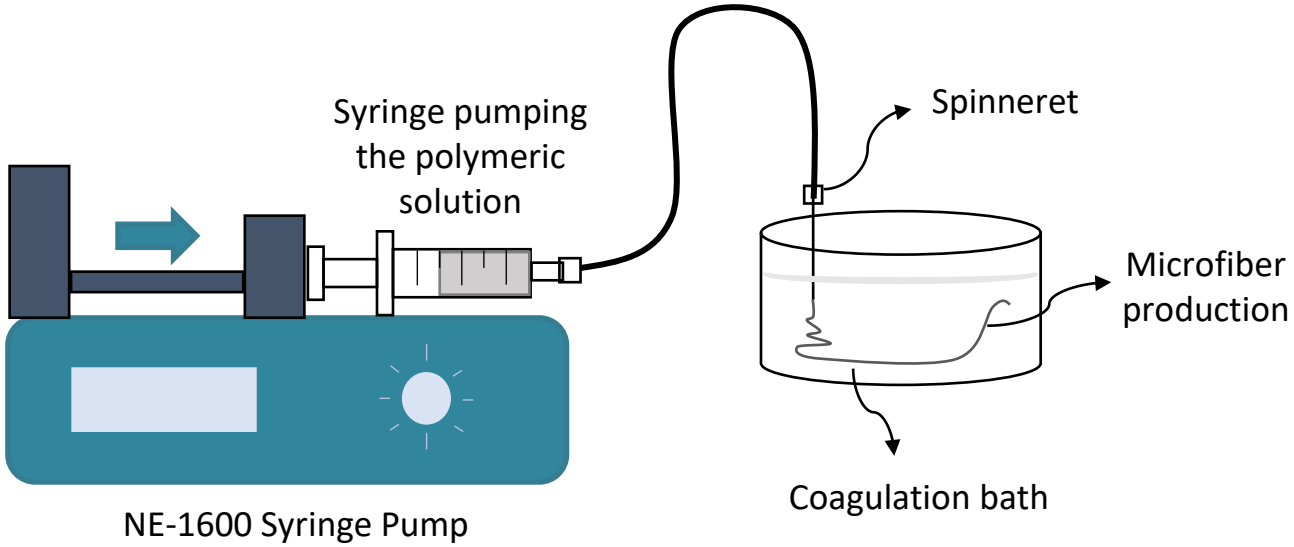
Tavares et al., *Antibiotics* 2020, *9*, 314 - doi:10.3390/antibiotics9060314

Goal

Engineer a biodegradable microfibrous target-delivery platform for EOs, that overcomes these biomolecules limitations for applications in infection control.

Methods - Wet-Spinning

Non-solvent induced phase inversion approach that allows the production of continuous polymeric **microfibers, with a uniform morphology**, by injecting a polymer solution into a non-solvent coagulation bath that prompts the solidification of the extruded material.

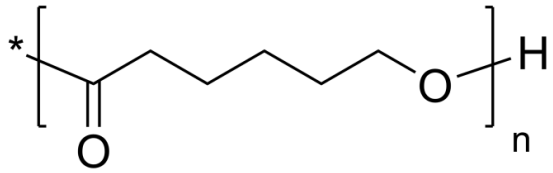


Felgueiras *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **634** 012033, doi:10.1088/1757-899X/634/1/01203

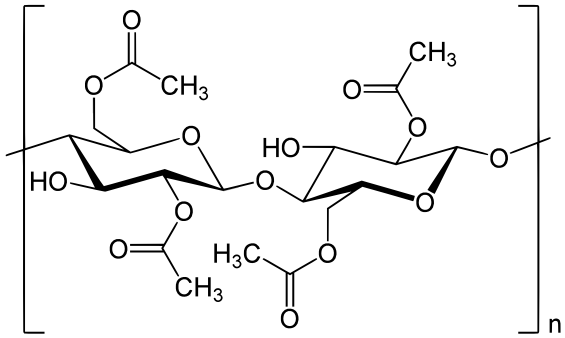
Methods - Microfiber Production

Biodegradable Polymers:

Polycaprolactone (PCL)



Cellulose Acetate (CA)



Polymeric solution preparation:

Solvents – acetic acid and acetone

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

Solubilization conditions – 1 h at 75 ºC and 200 rpm

Wet-spinning processing conditions:

Flow Rate – 0.5 mL/h

Needle Gauge – 18

Coagulation bath – Ethanol

Temperature of extrusion – 21 to 22 ºC

Results & Discussion - EOs Minimum Inhibitory Concentrations (MICs)

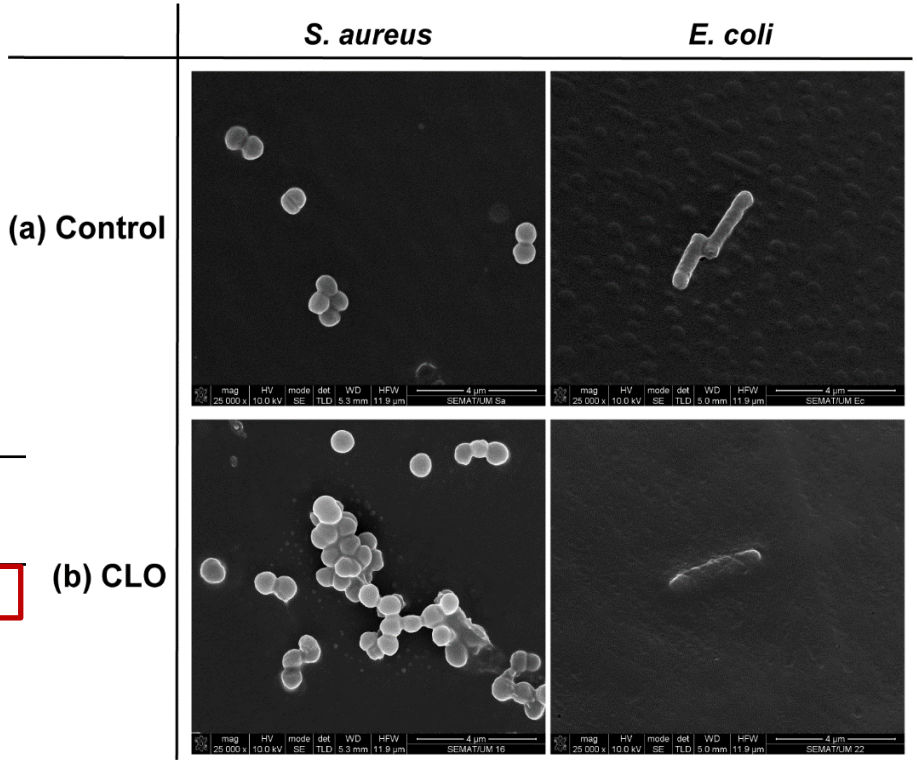
20 EOs with antimicrobial potential were examined for their MICs against the **Gram-positive *Staphylococcus aureus*** and the **Gram-negative *Escherichia coli*** bacteria, at initial concentration of $1x10^7$ CFUs/mL

Most effective:
Cinnamon leaf oil – CLO
Clove oil – CO
Cajeput oil – CJO

Control Antibiotic:
Ampicillin - A

Antimicrobial Agents	MICs (mg/mL) *S. aureus*	*E. coli*
CLO	0.82	0.82
CO	0.83	0.83
CJO	22.38	11.19
A	0.03	0.03

***SD < ± 0.5 mg/mL**



Tavares et al., *Antibiotics* 2020, *9*, 314 - doi:10.3390/antibiotics9060314

Results & Discussion - Loading Efficiency

EOs Incorporation:
Substrate – CA/PCL microfibers
Solvent – Ethanol
EOs concentration – 2 x MIC value
Conditions – 72 h at RT and 200 rpm, protected from light

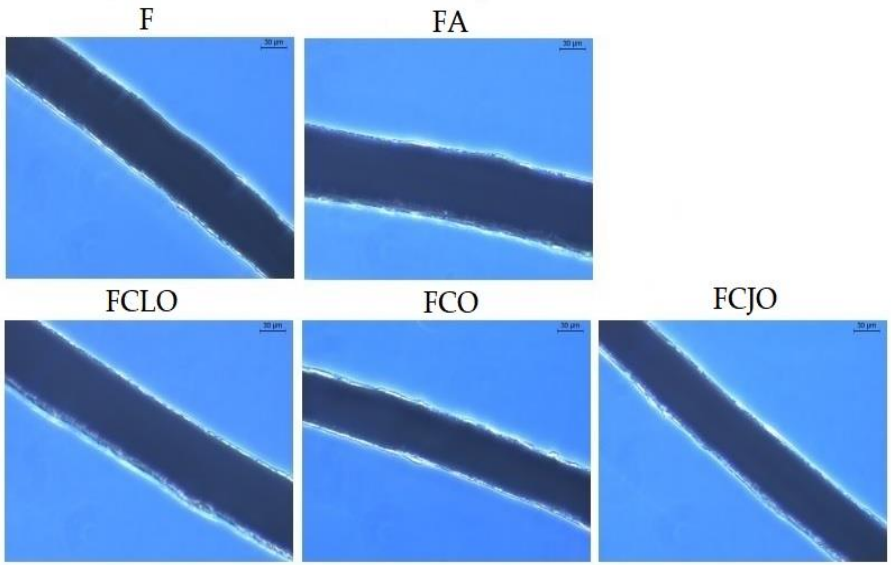
Loading Efficiency:
(mapped by UV-vis spectroscopy at 280 nm)

Antimicrobial Agents	Loading (MIC %, SD < ± 3.0%)	Concentration (mg/mL)
CLO	14.42	0.12
CO	66.08	0.55
CJO	76.48	17.12
A*	106.37	0.03

*Ampicillin was used as control to determine the maximum period for immobilization.

Results & Discussion - Fiber Morphology

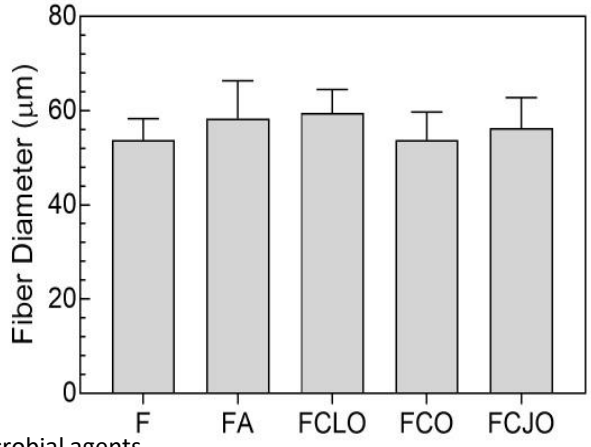
Microfibers Observation:
Brightfield microscopy
40x Magnification (30 µm scale bar)



Fibers presented a **uniform, homogeneous morphology**, free from defects.

Fibers Diameters:
Averaged from 40 measurements

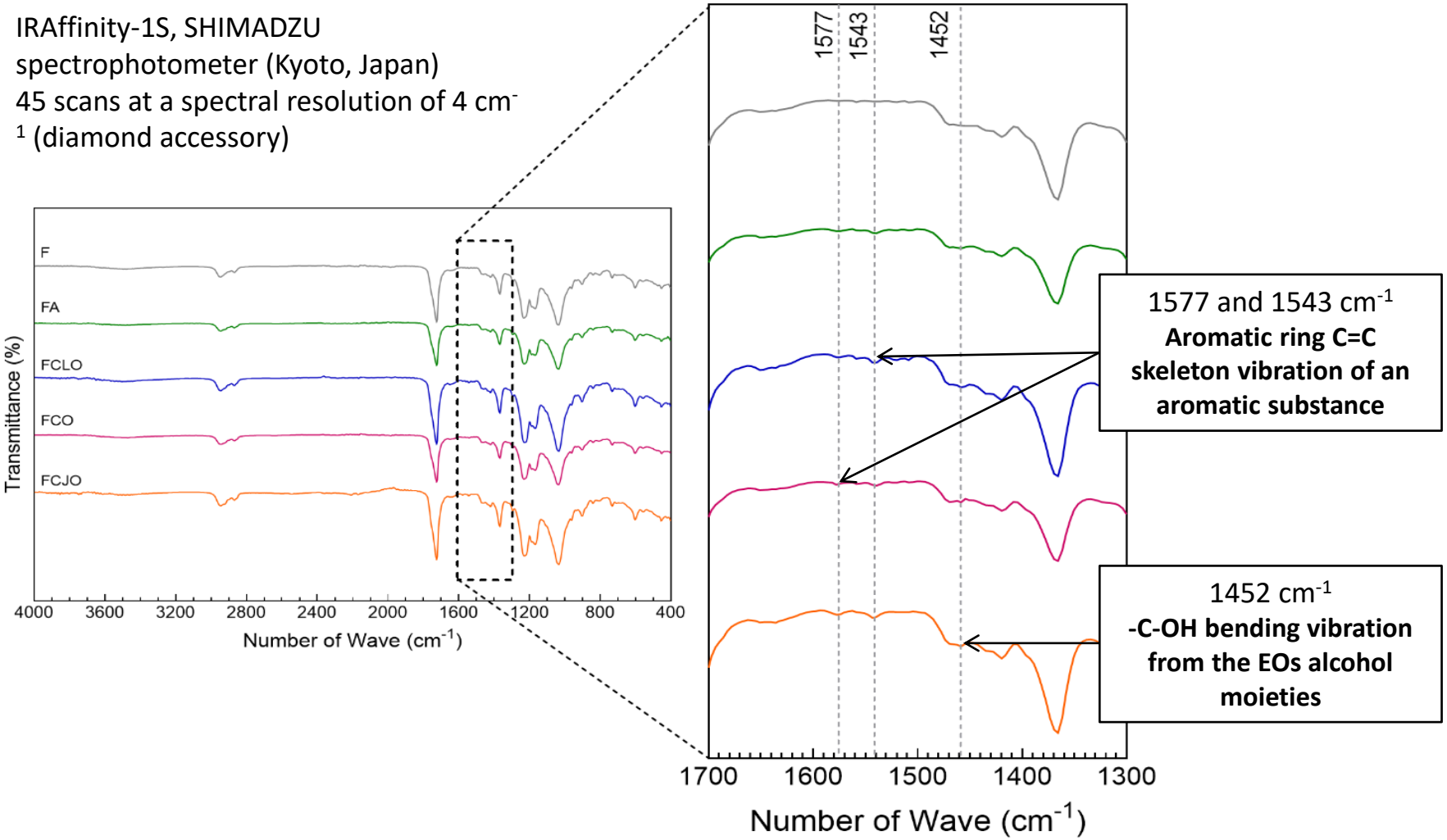
Diameters ranged between **54-59 µm**.



* F – unloaded microfiber; FA, FCLO, FCO and FCJO – microfibers loaded with antimicrobial agents

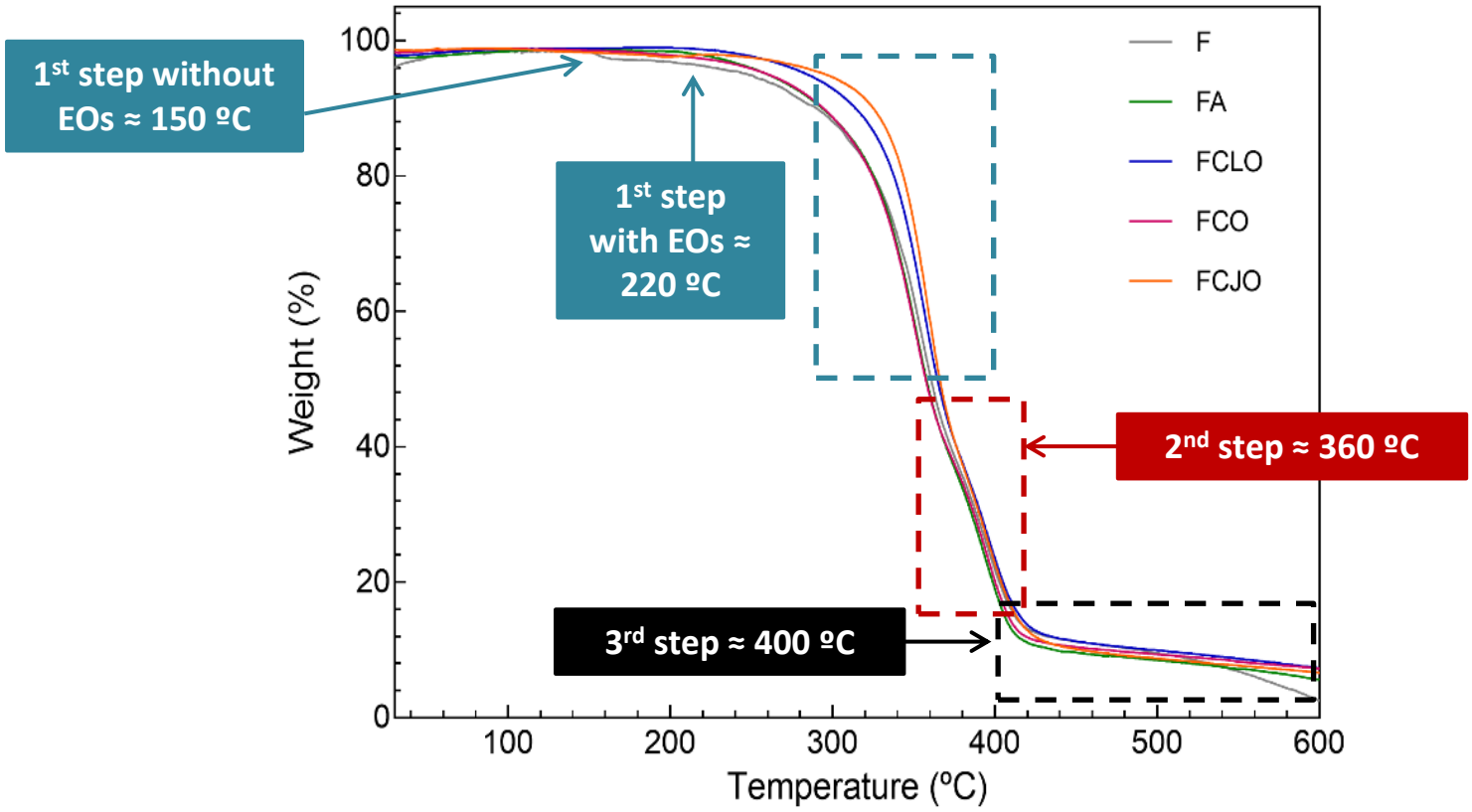
Results & Discussion - Chemical Characterization

IRAffinity-1S, SHIMADZU spectrophotometer (Kyoto, Japan)
45 scans at a spectral resolution of 4 cm^{-1} (diamond accessory)



Results & Discussion - Thermal Stability

STA 7200 Hitachi® (Fukuoka, Japan) with platinum pan
N_2 atmosphere, flow rate of 200 mL/min and T rise of 20ºC/min



Results & Discussion - Antimicrobial Action: Killing Time Kinetics

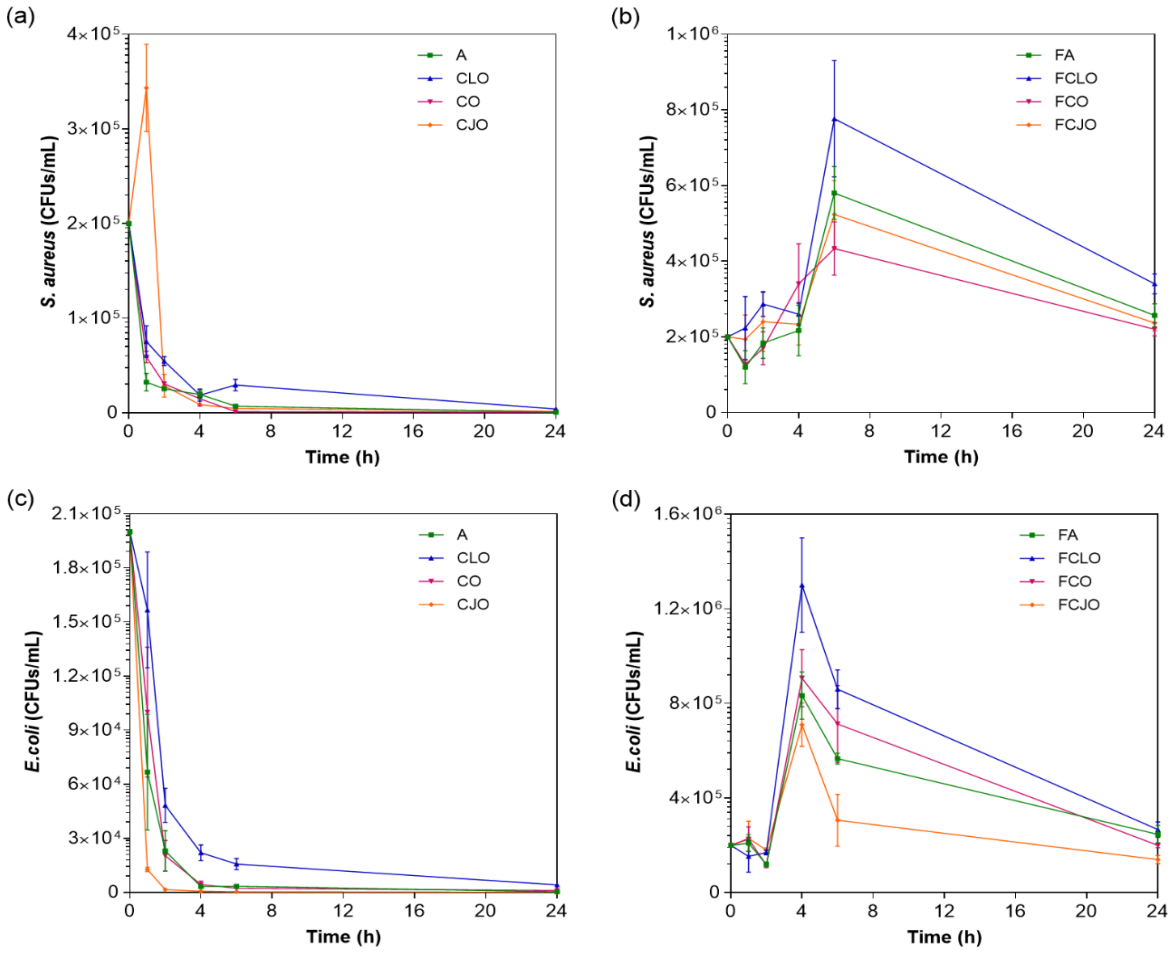
Bacteria Concentration:
1x10^5 CFUs/mL

Growth Conditions:
37°C and 120 rpm.

Incubation Periods:
0, 1, 2, 4, 6 and 24 h

Bacteria reduction was observed from the first moments of interaction.

Free EOs were more effective than loaded.



Results & Discussion - Antimicrobial Action: Killing Time Kinetics

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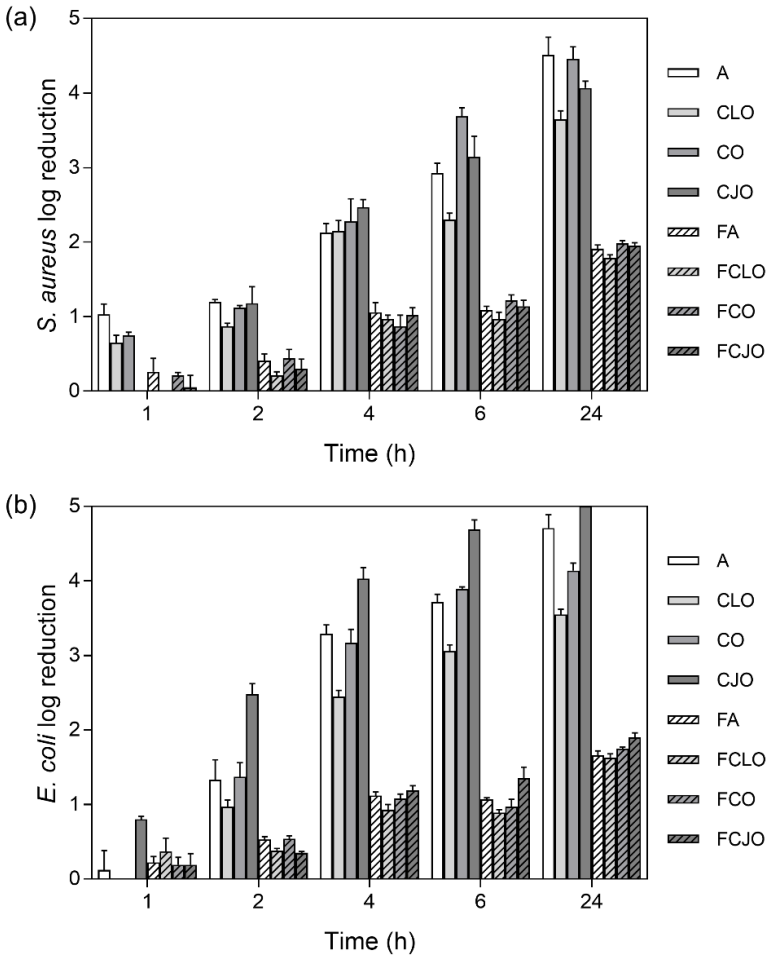
Incubation Periods:
0, 1, 2, 4, 6 and 24 h

Log reduction was most significant after 24 h of culture. At this point, it was evident that ***S. aureus* was more susceptible to the prolonged action of the EOs than the *E. coli*,** the only exception being the CJO.



Results & Discussion - Antimicrobial Action: Membrane Permeability

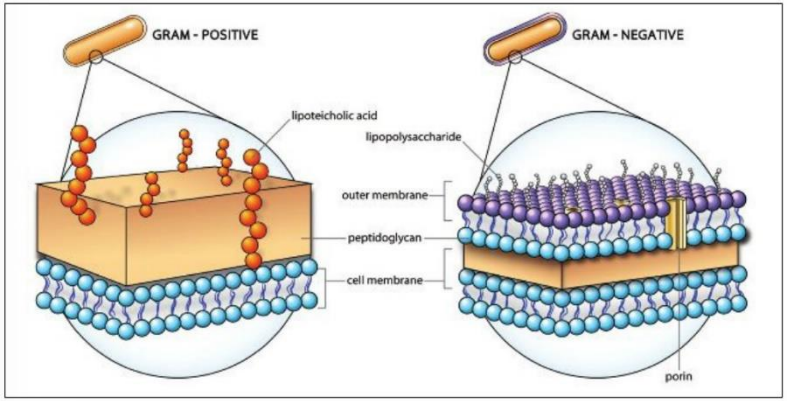
Bacteria Concentration: 1x10^5 CFUs/mL (adjusted in 5% glucose)
Growth Conditions: 37˚C and 120 rpm for 6 h
Conductance of bacteria suspensions can be used to examined the cell membrane penetration by antimicrobial agents.

Relative electric conductivities (%)

Bacteria	C	A	CLO	CO	CJO	F	FA	FCLO	FCO	FCJO
S. aureus	-0.49	19.10	9.81	19.83	20.41	-0.29	1.96	1.10	3.19	8.34
E. coli	-1.01	15.50	6.08	12.69	25.09	-1.32	0.75	0.67	1.69	4.49

Free state

Loaded



https://www.ddw-online.com/therapeutics/p320363-tackling-multi-drug-resistant-bacteria.html

Conclusions

EOs were successfully immobilized onto CA/PCL wet-spun fibers;

Microfibers displayed a **uniform and homogeneous** morphology with little variations in diameter;

FTIR and TGA data confirmed the successful incorporation of the EOs within the fibers by detecting characteristic peaks of the EOs and by demonstrating the increased overall **thermal stability** of the polymeric blend, respectively;

Even at small amounts, below MIC value, the **EOs-modified microfibers promoted cell death** compared to the control groups (unloaded and ampicillin-modified fibers), by disrupting and permeabilizing the cell cytoplasmic membrane;

The results demonstrated the potential of CA/PCL wet-spun microfibers loaded with EOs for applications in biomedicine, in which treatment of infections are a main target.

Full paper at: https://doi.org/10.3390/biom10081129
Felgueiras, H.P., et al. Biomolecules 2020, 10, 1129

Acknowledgments

2C2T Group

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Thank you for your attention.

COMPETE
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