Universidade do Minho
Escola de Engenharia

CENTRO DE CIÊNCIA E TECNOLOGIA TÊXTIL

# Wet-spun cellulose acetate/polycaprolactone fibers modified with essential oils for infection control

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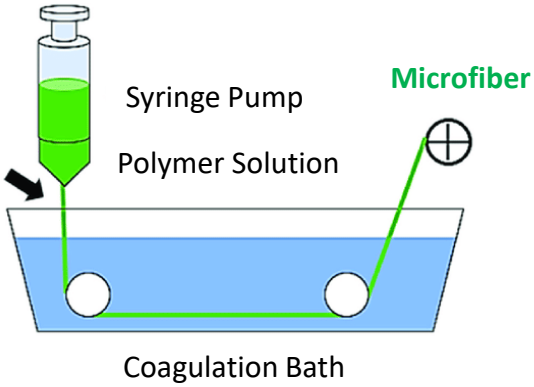
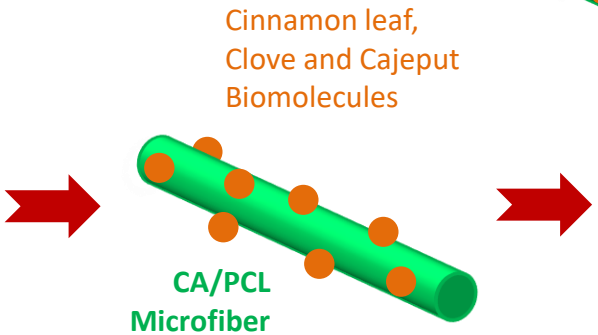
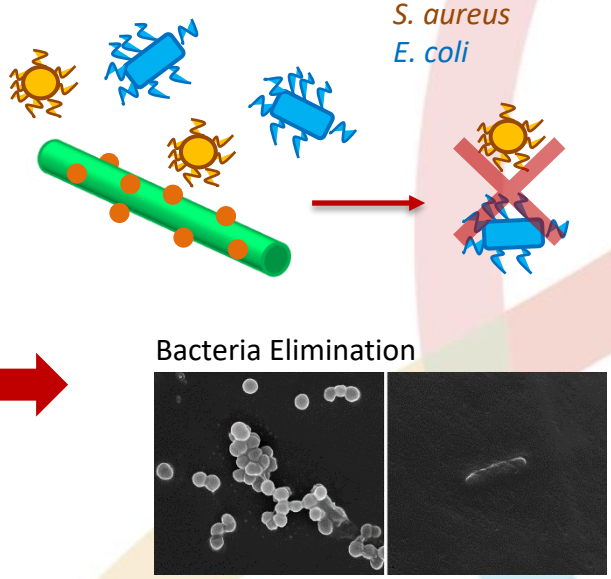
# Graphical Abstract




**Production of Wet-Spun CA/PCL Microfibers**




**Essential Oils Loading (Physical Binding)**

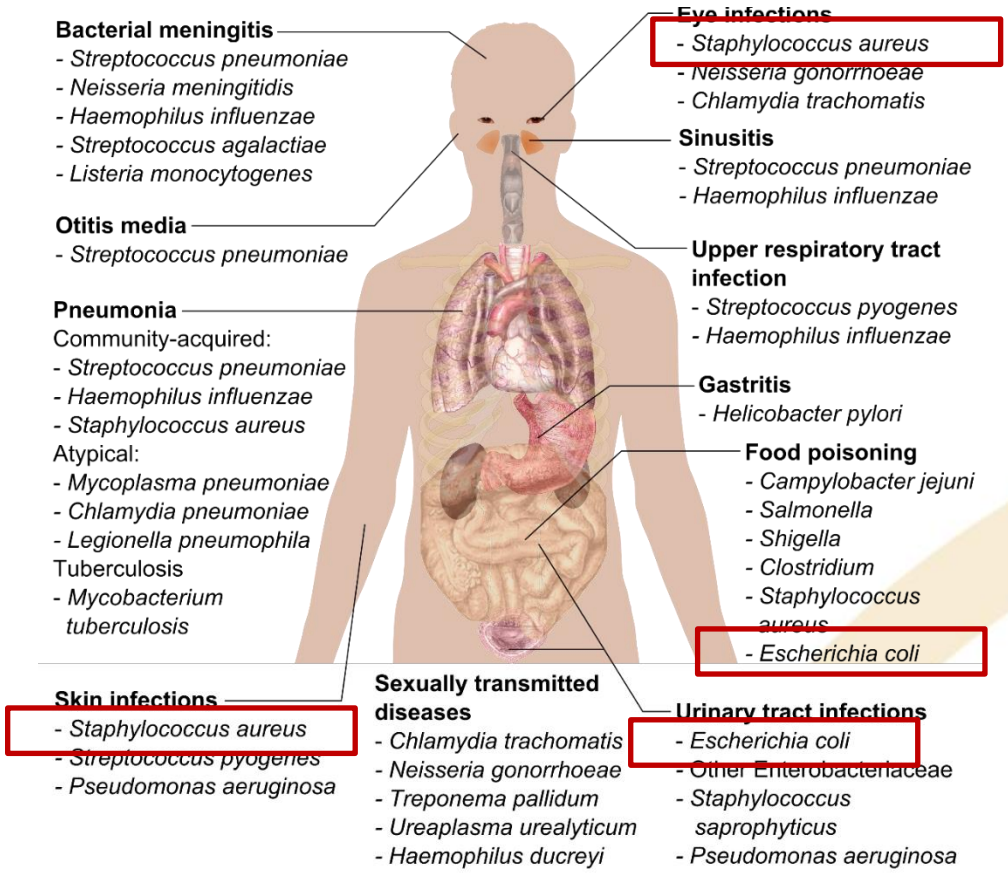



**Confirmation of Antimicrobial Efficiency**

(Representation not to scale)

## 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 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)

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

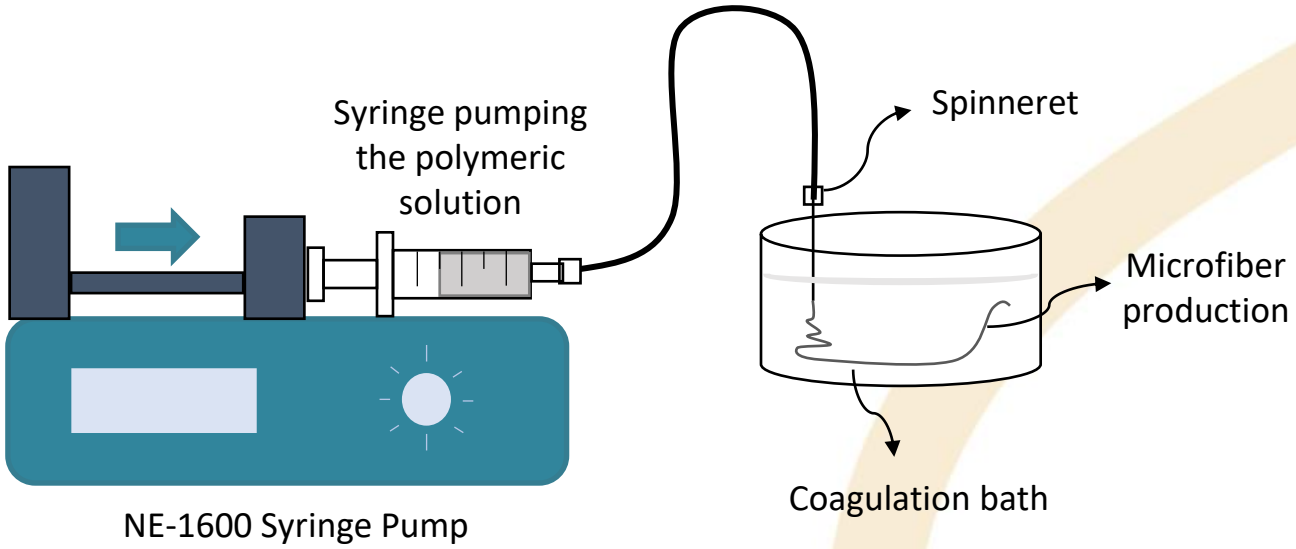
# Goal

**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.



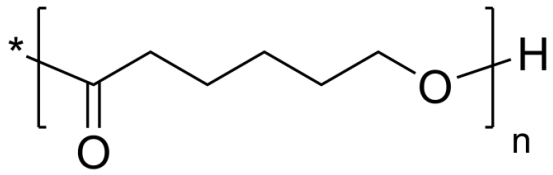

Felgueiras *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **634** 012033, doi:10.1088/1757-899X/634/1/01203

## Methods

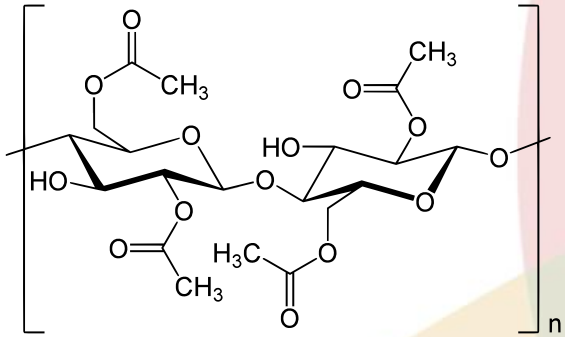
# Microfiber Production

**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 Concentration

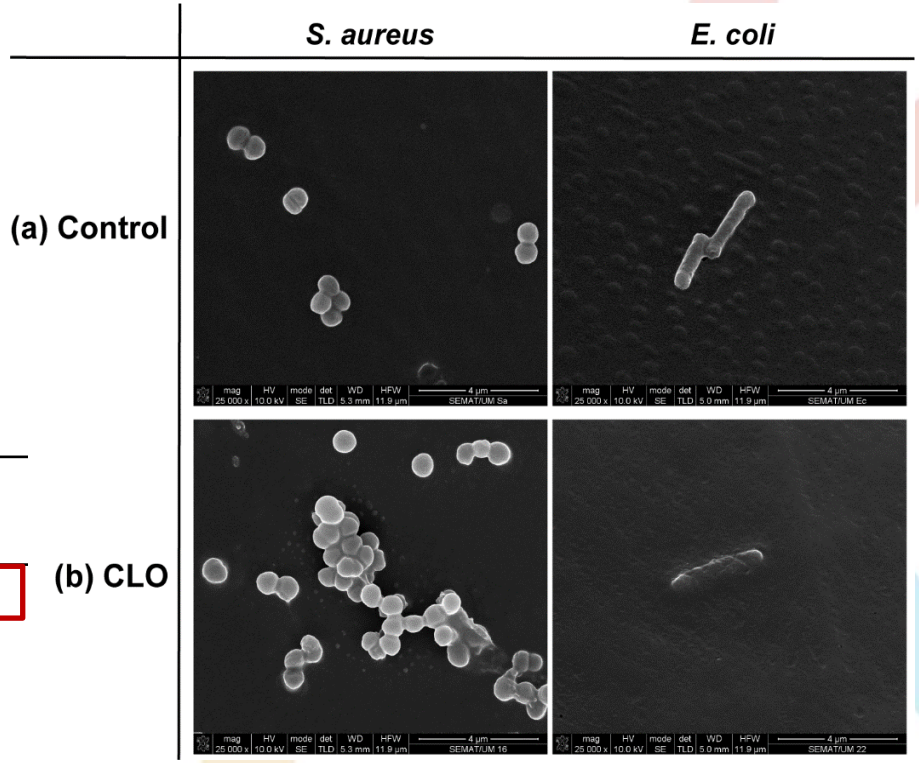
**20 EOs** with antimicrobial potential were examined for their minimum inhibitory concentrations (MICs) against the **Gram-positive *Staphylococcus aureus*** and the **Gram-negative *Escherichia coli*** bacteria, at initial concentration of $1x10^7$ CFUs/mL

**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)

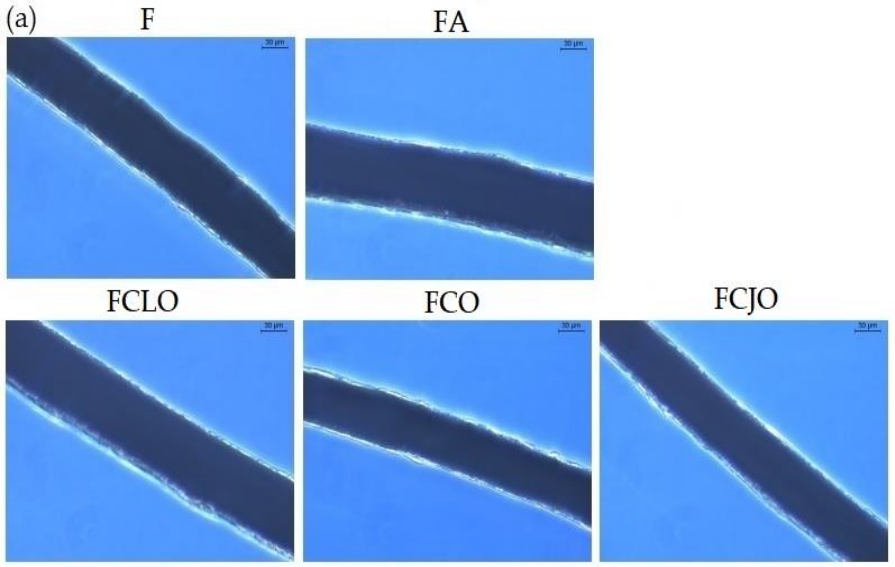
| Antimicrobial Agents | Loading (MIC %, SD < ± 3.0%) | Concentration (mg/mL) |
|---|---|---|
| CLO | 14.42 | 0.12 |
| CO | 66.08 | 0.55 |
| CJO | 76.48 | 17.12 |
| A* | 106.37 | 0.03 |

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

## Results & Discussion

# Fiber Morphology

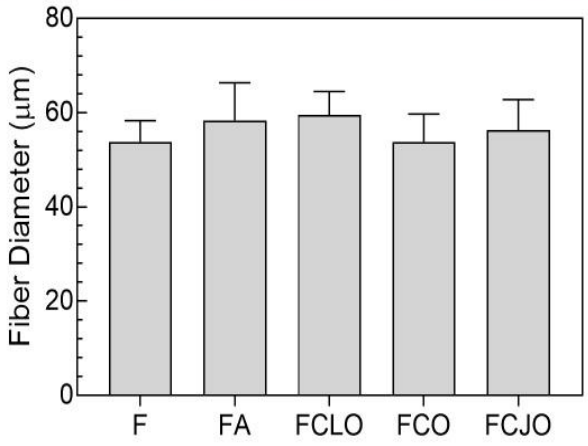
**Microfibers Observation:**
Brightfield microscopy
40x Magnification (30 µm scale bar)




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

**Fibers Diameters:**
Averaged from 40 measurements

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

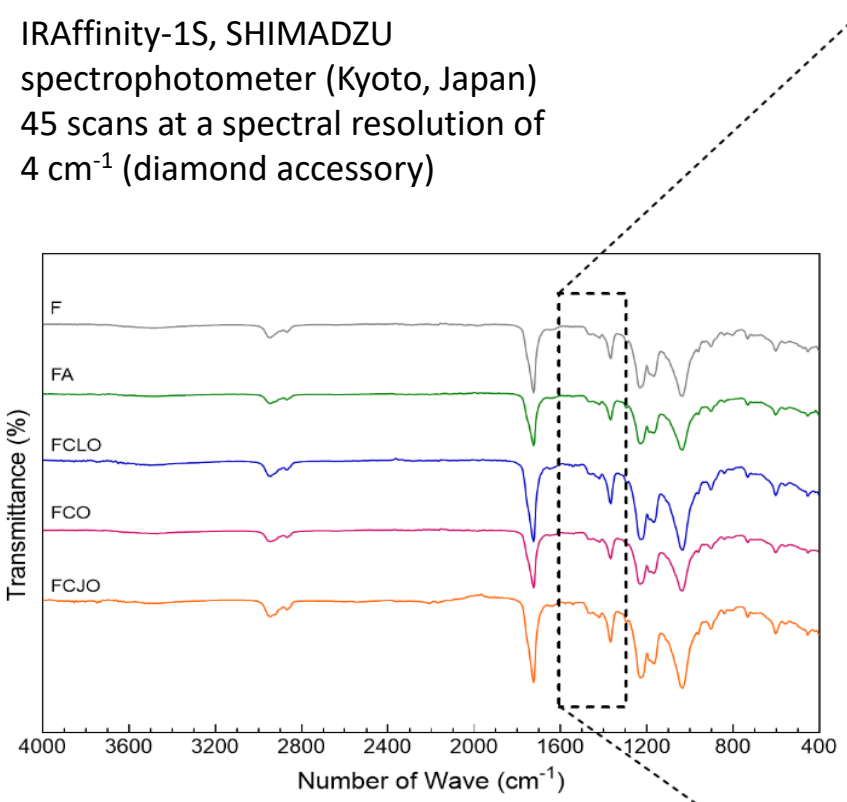


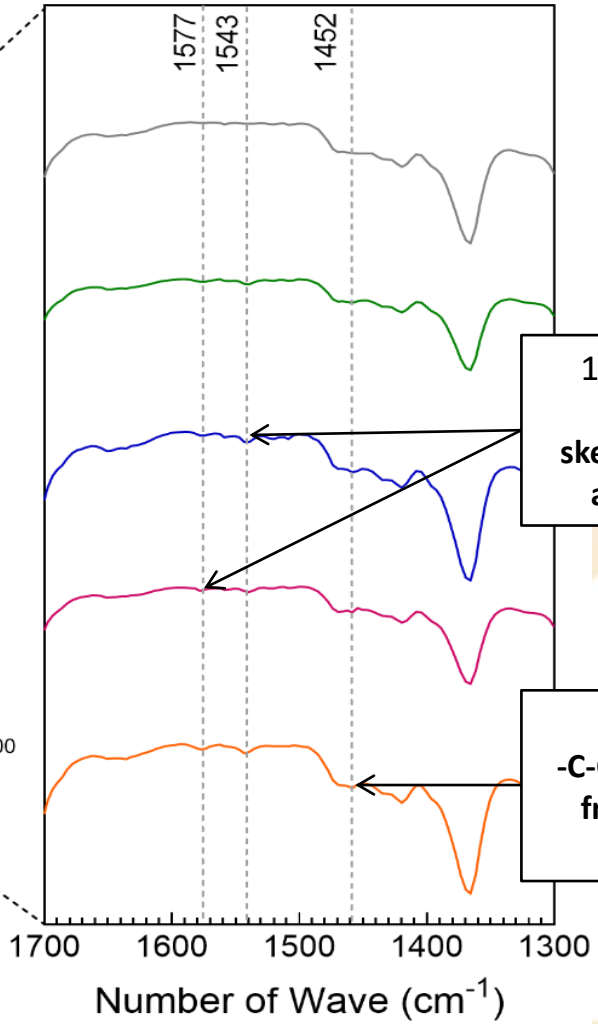

* F – unloaded microfiber; FA, FCLO, FCO and FCJO – microfibers loaded with antimicrobial agents

## Results & Discussion

# Chemical Characterization

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



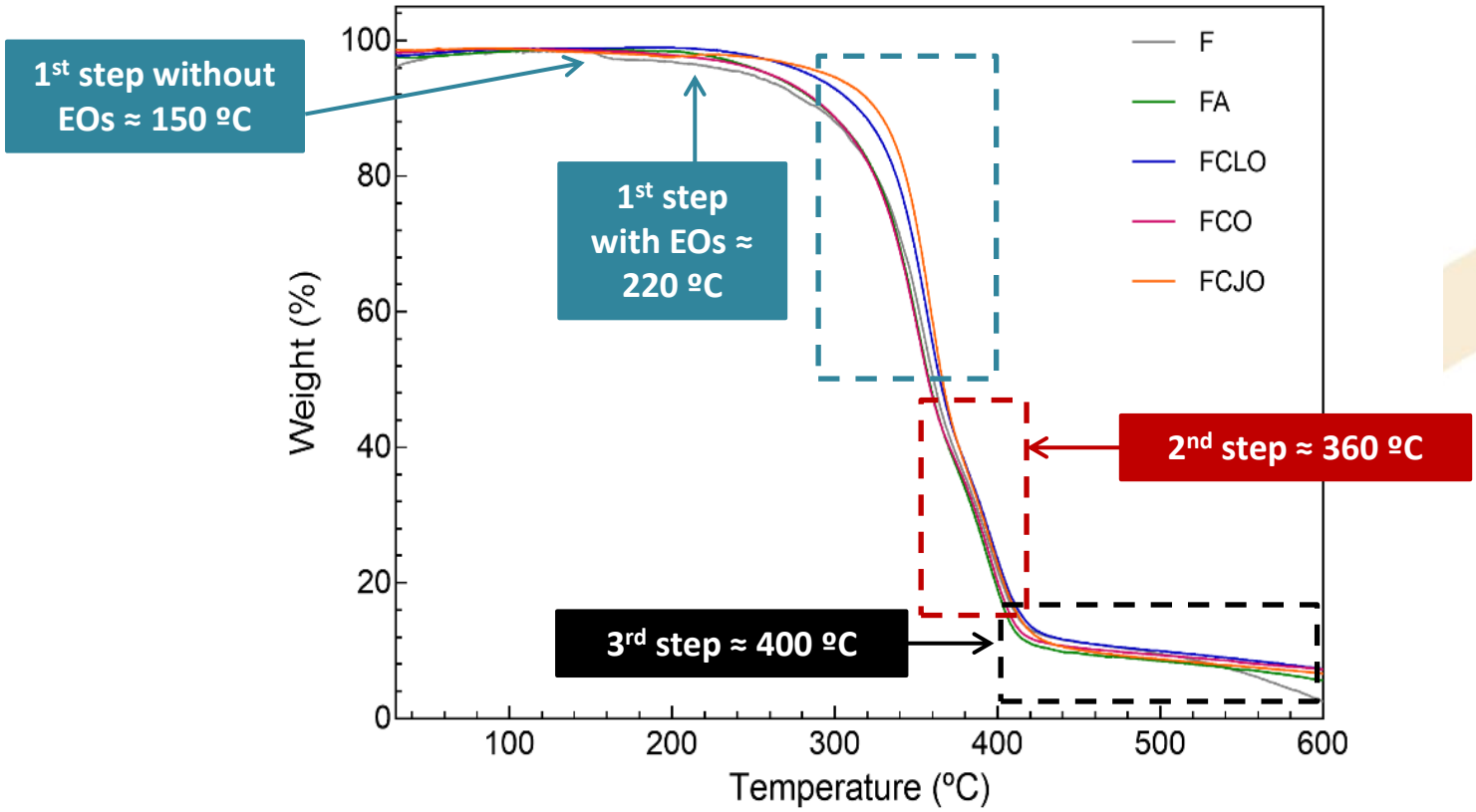

1577 and 1543 $cm^{-1}$
**Aromatic ring C=C skeleton vibration of an aromatic substance**

1452 $cm^{-1}$
**-C-OH bending vibration from the EOs alcohol moieties**

## 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

# Time Kill Kinetics

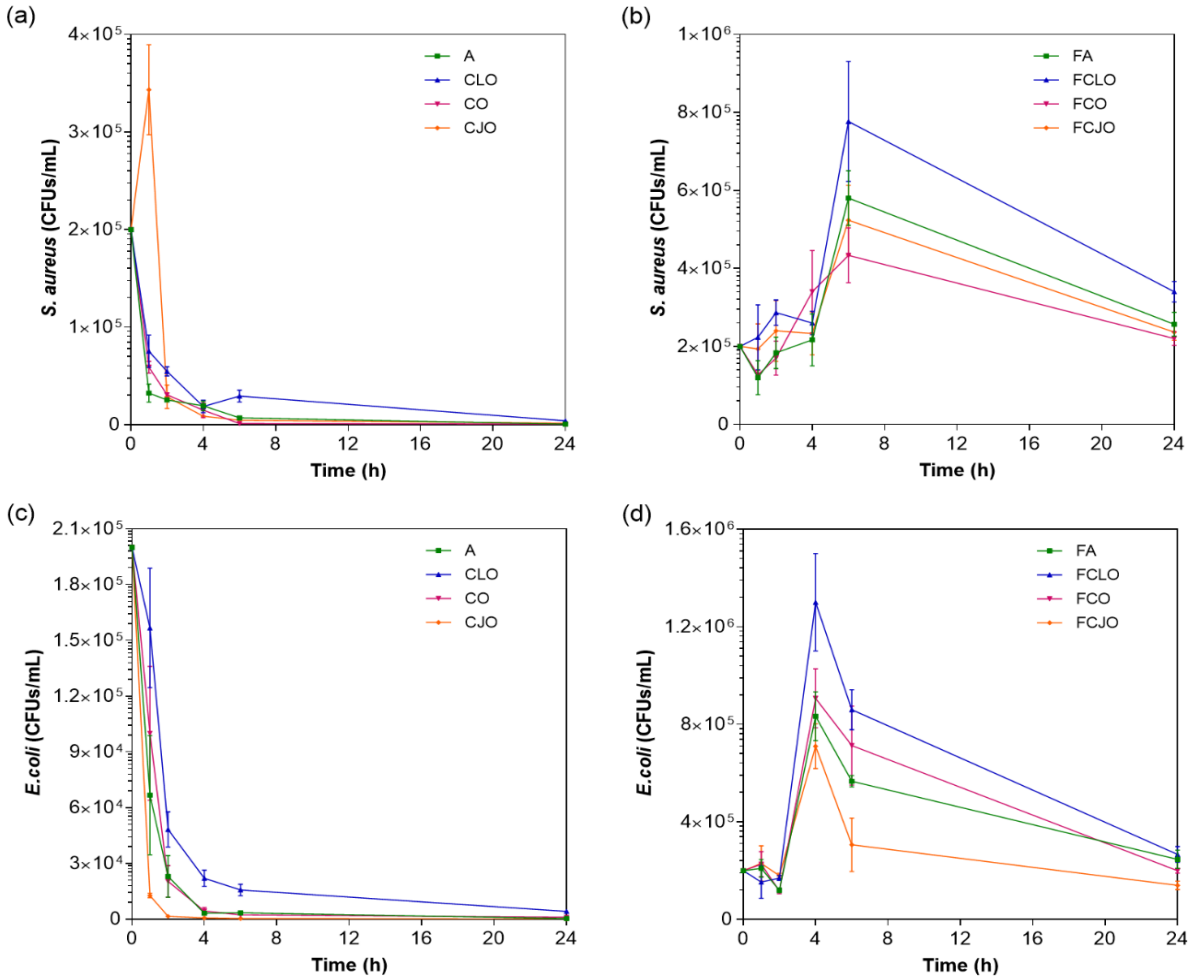
**Bacteria Concentration:**
1x10$^5$ CFUs/mL

**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.**



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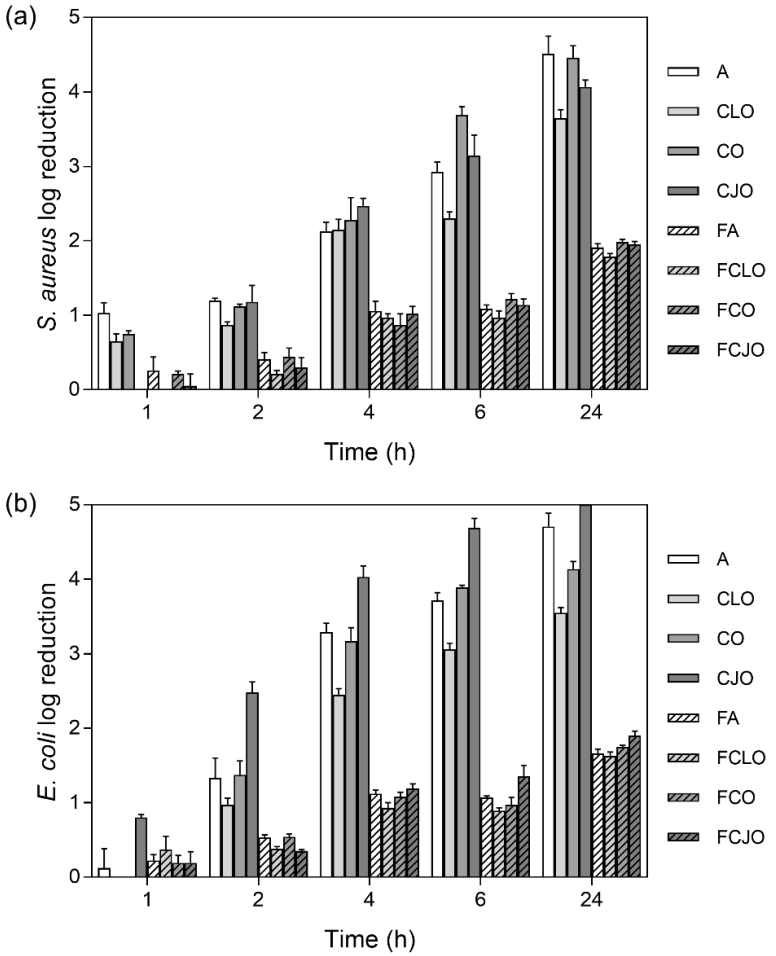



Log reduction was most significant after 24 h of culture. At this point, it was evident that ***S. aureus* was more susceptible to the prolonged action of the EOs than the *E. coli*,** the only exception being the CJO.

## Results & Discussion: Antimicrobial Action

# Membrane Permeability

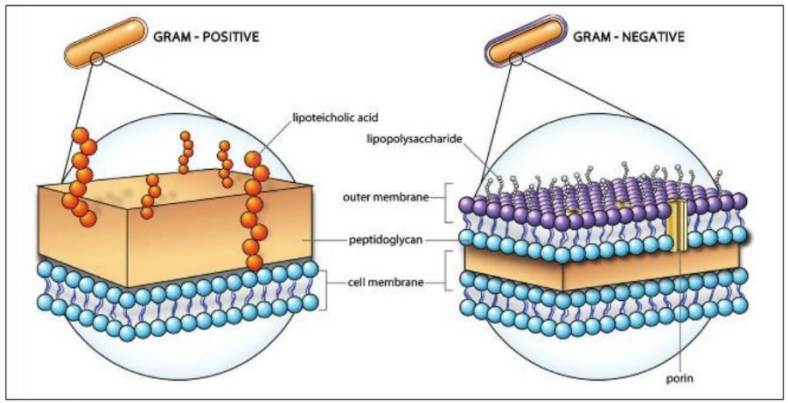
**Bacteria Concentration:** $1x10^5$ CFUs/mL (adjusted in 5% glucose)
**Growth Conditions:** 37°C and 120 rpm for 6 h
Conductance of bacteria suspensions can be used to examined the cell membrane penetration by antimicrobial agents.

**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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