Universidade do Minho
Escola de Engenharia

CENTRO DE CIÊNCIA E TECNOLOGIA TÊXTIL

Essential oils-loaded nanofibrous mats inhibitory effect against the *Escherichia* virus MS2, mimic of SARS-CoV-2

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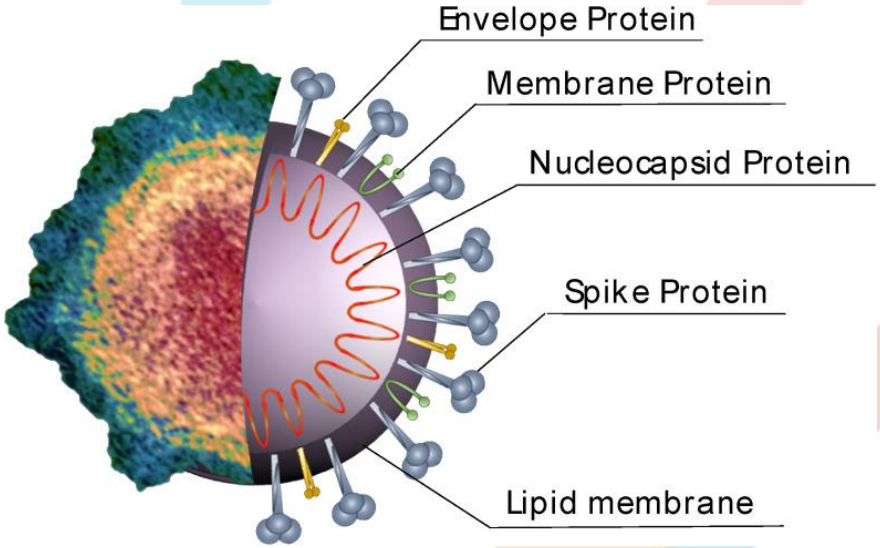
COVID-19

In December 2019, a novel strain of coronavirus, SARS-CoV-2, was identified. Infected patients revealed symptoms of fever, cough (dry), sore throat, and fatigue, which began manifesting after 5 days of incubation.



Hoping to prevent transmission, many countries adopted a mandatory mask use. However, most mask options display a passive action against COVID-19.

Essential Oils (EOs)

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

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Biomolecules endowed with **antimicrobial and regenerative potential**

⇩

Anti-Viral ⇨

Example: **garlic oil** is capable of inhibiting the angiotensin-converting enzyme 2 protein, leading **SARS-CoV-2** to lose its host receptor and attack the PDB6LU7 protein (its main protease), thus preventing virus maturation and spreading.

EOs are also known **for relieving symptoms** of cough and fever associated to **respiratory illnesses**.

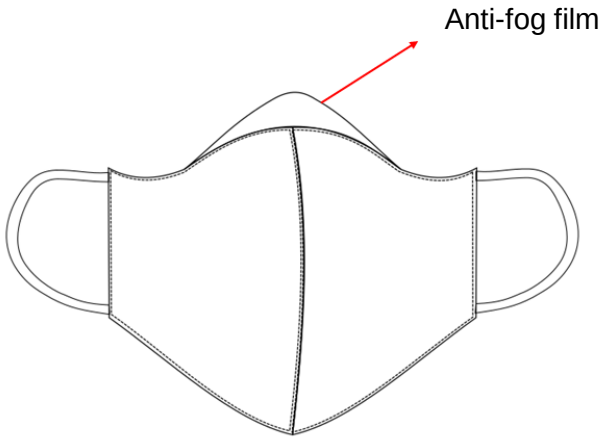
Tavares et al., Biomolecules 2020, 10, 148- doi:10.3390/biom10010148; Bui Thi Phuong Thuy et al., ACS Omega 2020, 5, 14, 8312−8320.

Goal

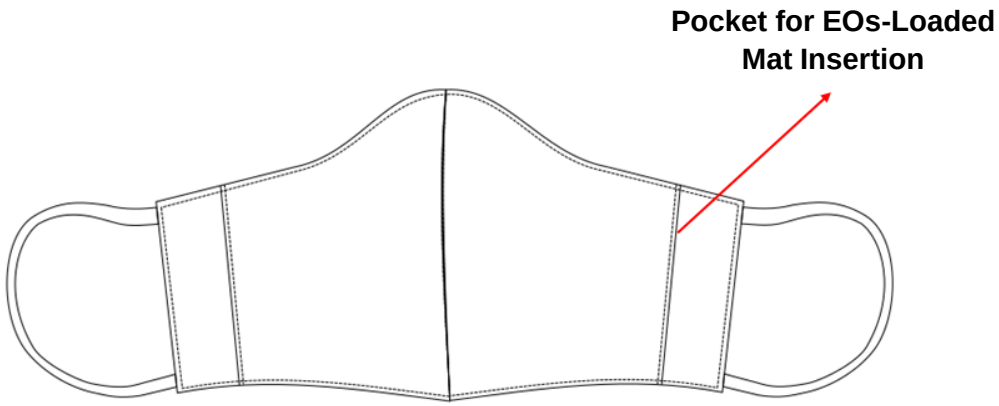
This work proposes the incorporation of anti-viral EOs on a nanofibrous mats that can be adapted to both hand-made and commercial masks.

Mask Schematics

Example: re-usable mask



Front-side



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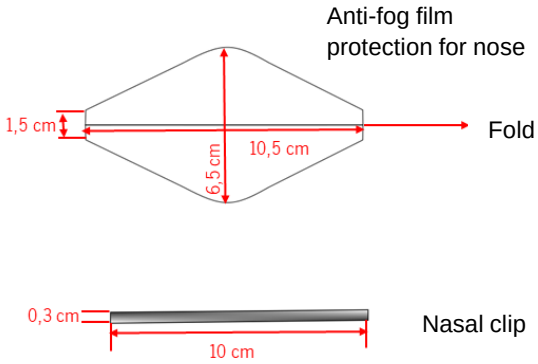
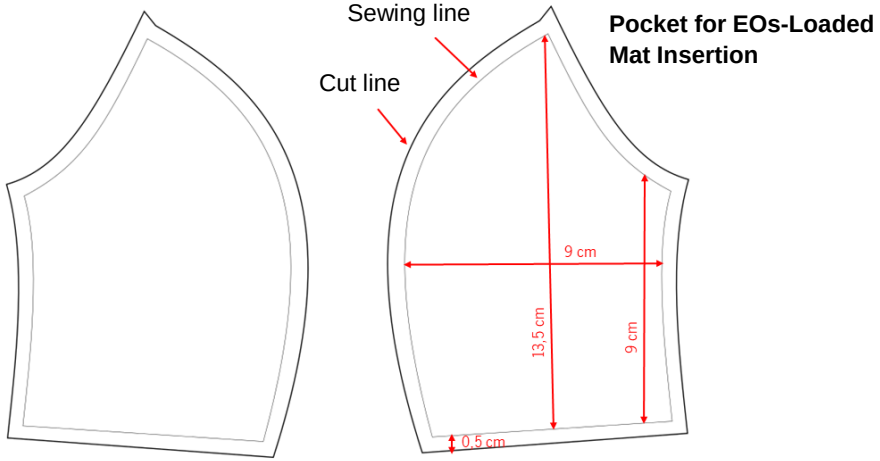




Modeling of the Pocket for EOs-Loaded Mat Insertion

EOs Selection

20 EOs with antimicrobial potential were examined for their minimum inhibitory concentrations (MICs) against the MS2 *Escherichia* host and for their virucidal concentration (VC) against the MS2 virus, mimic of SARS-CoV-2, at initial concentration of $1x10^7$ CFUs or PFUs/mL, respetivelly.

List of tested EOs:
Amyris; Cajeput; Cinnamon leaf; Citronella; Clove; **Eucalyptus**; Frankincense; Geranium; Himalayan cedar; Lavandin; **Lemongrass**; **Niaouli**; **Orchid**; Palmarosa; Patchouli; Rosemary; **Sage**; Star anise; **Tea tree oil**; Wintergreen.

EOs	MICs against MS2 host (mg/mL)*	VC against MS2 virus (mg/mL)*
Lemongrass (LGO)	178.0	**356.0**
Niaouli (NO)	45.7	**365.2**
Eucalyptus (ELO)**	>	**586.0**
Orchid	85.6	428.0
Tea Tree Oil	22.4	447.5
Clove	105.6	528.0

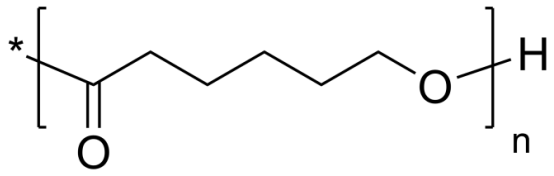
* Standard deviation was < ± 5.0 mg/mL.
**Even though the VC is superior to the last three-placed EOs, the amount of oil required for loading is inferior due to its increased density.

Felgueiras et al., *Biomolecules* 2020, 10, 1129- doi:10.3390/biom10081129

Electrospun Nanofibers

Selected polymer

Polycaprolactone (PCL)



Why PCL?
- Food and Drug Administration (FDA)-approved;
- Drug loading efficiency (i.e., EOs biomolecules);
- Excellent mechanical strength, non-toxic, hydrophobic, biodegradable, etc.

Polymeric solution preparation
Concentration: 14 wt.%
Solvents: chloroform/dimethyl formamide (CHF/DMF at 9/1 v/v)

Electrospinning processing conditions
Potential: 23 kV
Extruding Speed: 0.7 mL/h
Distance to Collector: 26 cm
Needle (inner diameter): 18 gauge

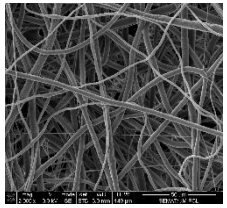
Felgueiras et al., *Biomolecules* 2020, 10, 1129- doi:10.3390/biom10081129

Nanofibers Loading

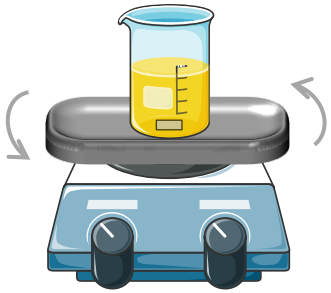
Loading Amount: **10% of VC**
(> 10% VC turned the mats sticky and the fragrance too intense)

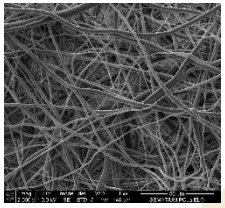
Strategy 1

Physical adsorption of the EOs at the surface of the electrospun PCL mats (label **PCLa**EOs)



24 h immersion of PCL electrospun mats within EOs solutions, at 150 rpm (orbital shaking)



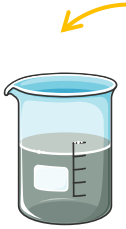


Mats' ethanol washing and subsequent drying

Strategy 2

Blending the PCL solution with the selected EOs (label **PCLb**EOs)

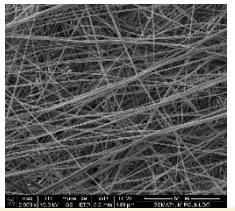
PCL dissolution in CHF/DMF for 24 h



Addition of EOs and continued blending for 24 h

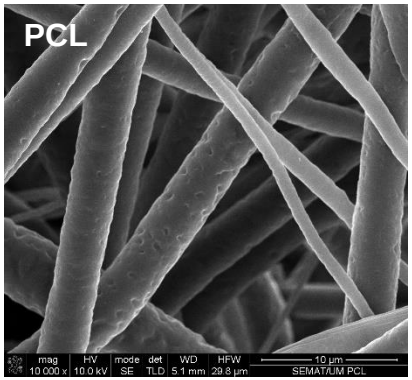
PCL blend loaded with EOs

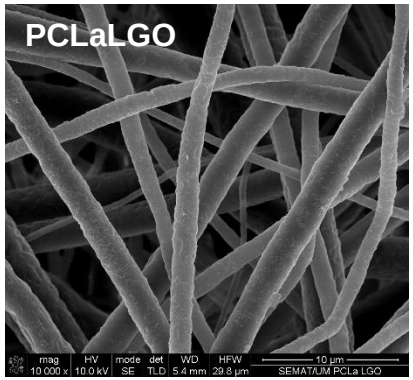
Electrospinning

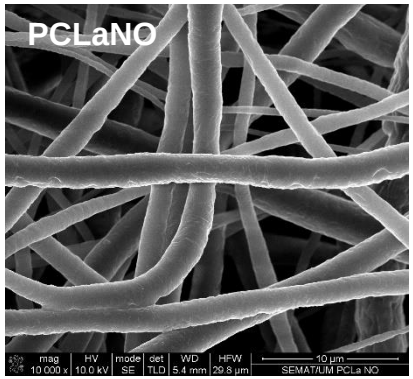


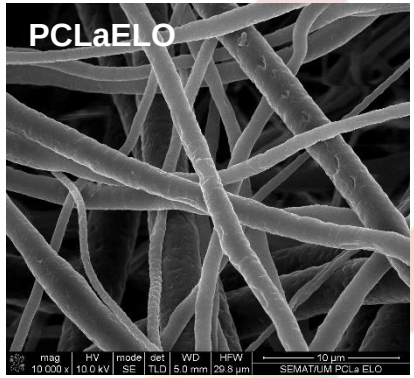
Nanofiber Morphology

Scanning electron microscopy (SEM) observations
10 000x magnification



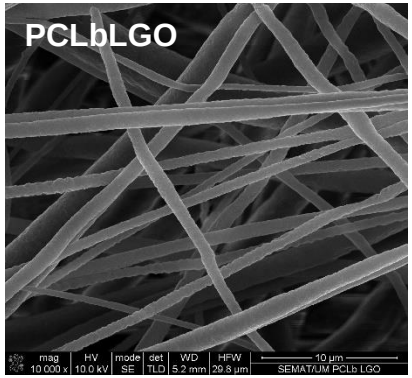


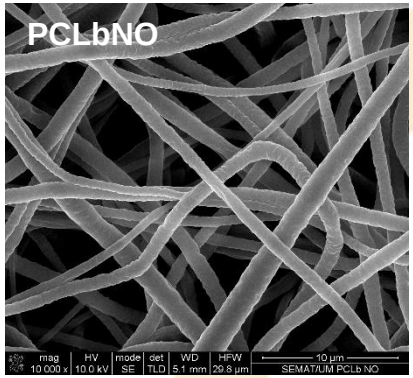


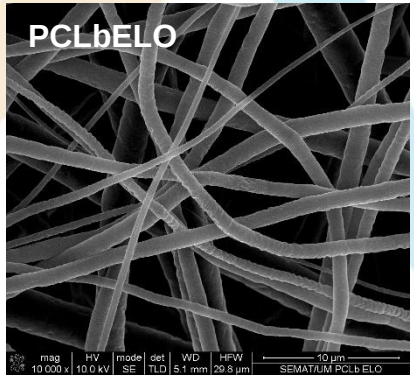


Nanofibers are **uniform and continuous (defect free)**.

EO adsorption does not alter the fibers diameters. **Blending PCL with EOs before electrospinning reduces the average diameter of the fibers and increases porosity.**







Loading and Release

EOs Loading: UV-visible spectroscopy

- PCLaEOs mass determinations made indirectly, via EOs immersion solutions (10% VC) and ethanol washing bath
- PCLbEOs mass determinations made directly on the modified mats

EOs release: solid phase micro-extraction followed by gas chromatography-mass spectrometry (SPME-GC-MS)

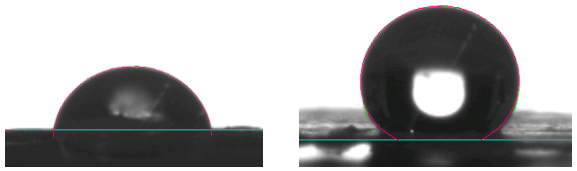
- Normalized to the most important compound within the EOs, namely citral (LGO) and 1,8-cineole (NO and ELO).
- 4 h testing at 35ºC (skin temperature)

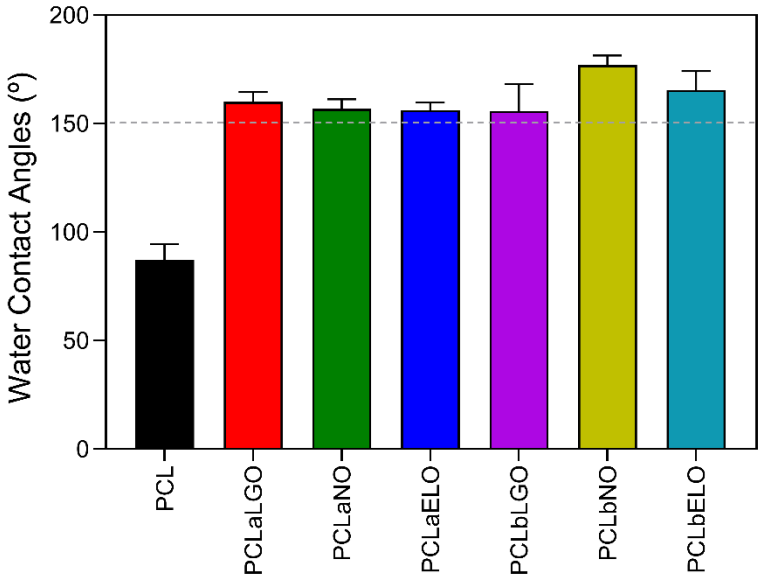
	Mats	Loaded Mass (µg)	EOs Release (%)
Strategy 1: Physical Adsorption	PCLaLGO	5257 ± 2510	50.3 ± 0.1
	PCLaNO	800 ± 184	0.3 ± 0.1
	PCLaELO	5079 ± 4122	0.1 ± 0.0
Strategy 2: Blending	PCLbLGO	1673 ± 641	13.3 ± 3.5
	PCLbNO	1128 ± 98	11.3 ± 8.4
	PCLbELO	631 ± 173	10.7 ± 6.3

Wettability

Contact Angle OCA 15, Data Physics (Filderstadt, Germany)
Standard Followed: ASTM-D7334-08, sessile drop method
Drop Size: 10 µL water drops (video-based drop shape analyzer, OCA15 plus software)

Without Oil **With Oil**



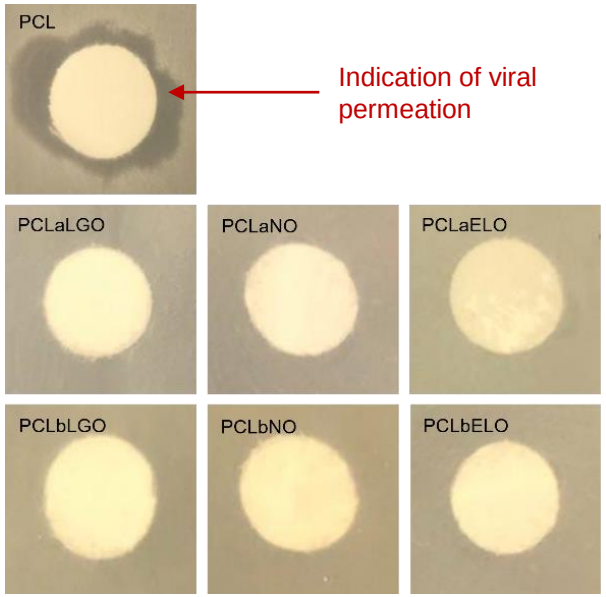


Addition of EOs increased the hydrophobicity of the mats **from hydrophobic to superhydrophobic (> 150º).**

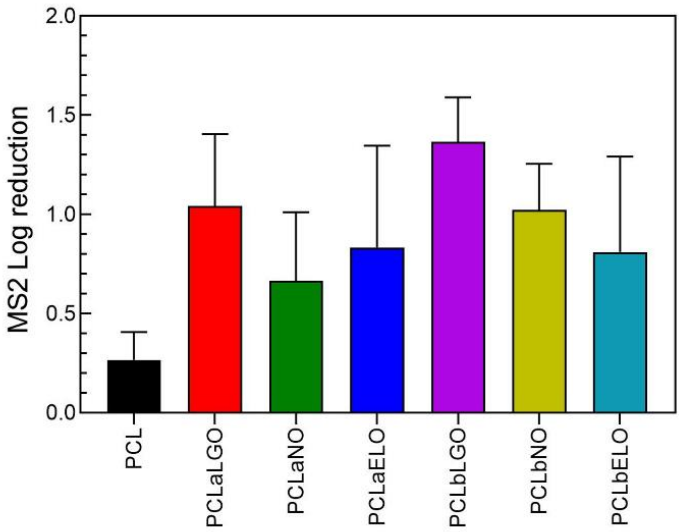
This **water-repellency** feature will prevent the virus from penetrating the mask.

Antiviral Performance

Contact Killing Examinations
Microorganism: Bacteriophage MS2 (*Escherichia coli* virus, mimic of SARS-CoV-2)
Initial Concentration (t = 0 h): 1.0 x 10^7 PFUs/mL
Culture Media: ATCC medium 271



Testing done with **5 µL** viral droplets



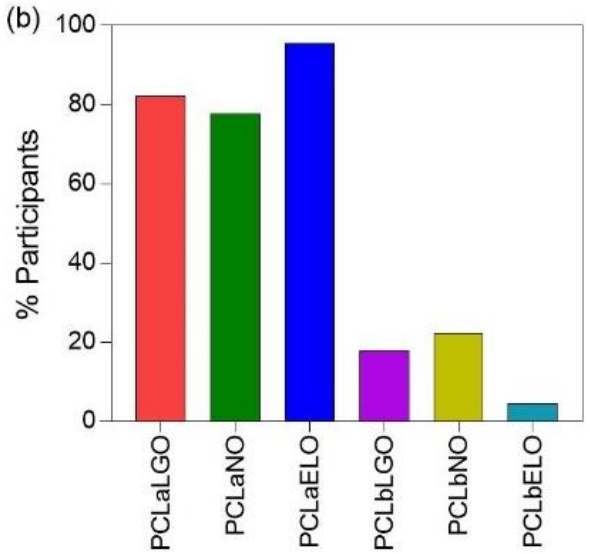
Testing done with **50 µL** viral droplets

MS2 reduction was observed after 4 h of interaction on all EO-loaded mats. **PCL blended with LGO was the most effective from the group in fighting the virus. EOs-loaded mats were also seen to retain virus and prevent penetration through mask.**

Sensory Testing

Descriptive, discriminatory and affective analyses were conducted

Number of participants: 45, aged between 18 and 59 years (average of 30 years) and made of 60% females and 40% males
Smelling was conducted at **1 to 2 cm distance from the face**, for at least 30 seconds



Participants found PCLaEOs more odor intense than PCLbEOs.

PCLbLGO was deemed the most pleasant from the group, while PCLaELO was considered the least.

Conclusions

Data demonstrated the potential of PCL electrospun fibers loaded with EOs to be used for prospective protection mask uses (i.e., as intermediate layers).

The most antiviral effective combination was the PCL blended with LGO, which was also deemed the most pleasant by the sensory testing participants.

Production Cost/EOs-Loaded Mat:

- LGO (100 mL) = 17.66 €
- PCL (500 g) = 304.00 €
- CHF (2.5 L) = 108.00 €
- DMF (5 L) = 204.10 €
- Energy costs = 0.04 €/h

5 mL of PCLbLGO and 2 h production = **0.71 € per Mat**

Lab-Scale Production

↓

Large-Scale Production Cost Projections

=

0.23 € per Mat

Acknowledgements

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