Universidade do Minho
Escola de Engenharia

CENTRO DE CIÊNCIA E
TECNOLOGIA TÊXTIL

Bactericidal action of plant-derived lipophilic drugs enclosed by marine-derived polymeric films

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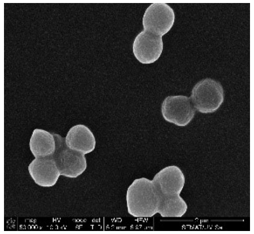
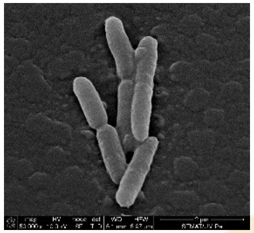
Infected wounds

Bacteria are primarily responsible for diabetic foot ulcer (DFU)'s infections, being *S. aureus* the most common bacteria isolated (46.4%), followed by *P. aeruginosa* (22.8%)

S. aureus is a Gram-positive, commensal bacterium



P. aeruginosa is a Gram-negative, invasive bacterium



The increased resistance of bacteria against antibiotics

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serious concerns about DFU therapeutic strategies

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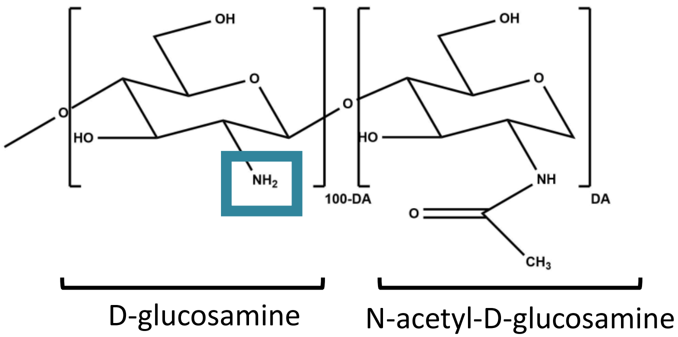
Bio-based treatments with quick bactericidal action
and low tendency to induce resistance are greatly needed.

Tavares, TD, Antunes, JC *et al.*, *Antibiotics* **2020**, *9*(6), 314

Antibacterial CS



It is suggested that the **antimicrobial activity of the** marine-derived polysaccharide **CS** results from **its cationic nature**

Antimicrobial mechanisms

- ✓ **Electrostatic interaction** between positively charged R-NH_3^+ sites and negatively charged microbial outer **cellular components** and/or cellular membrane leads to cellular impermeability (inhibiting growth) or cellular lysis (killing bacteria). CS internalization and interaction with cytoplasmic constituents may also occur
- ✓ **Chelation of metals**, **suppression of spore elements** and **binding to essential nutrients** to microbial growth interfere with their growth and may contribute to their death

CS's antimicrobial activity is **influenced** by **various intrinsic and extrinsic factors**

CS itself (type, Mw, DA, viscosity, solvent and concentration)

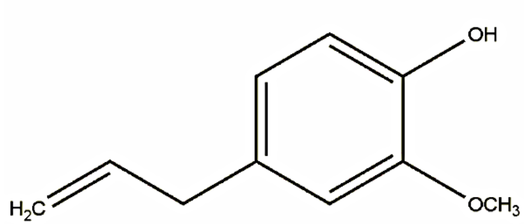
environmental conditions (test strain, its physiological state and the bacterial culture medium, pH, temperature, ionic strength, metal ions)

Antibacterial CLO and CO

Essential oils (EOs):

- ✓ aromatic, volatile, lipophilic biomolecules, extracted from regions of plants (e.g. flowers, leaves, twigs, bark, wood, fruits, etc.)
- ✓ formed of complex mixtures of hydrophobic molecules, including thymol, carvacrol and eugenol (among others), which exhibit a broad spectrum of antimicrobial activity against bacteria, fungi, and viruses
- ✓ potential to replace antibiotics due to their inherent and strong anti-inflammatory, antiseptic, analgesic, spasmolytic, anesthetic, and antioxidative properties

rich in eugenol →

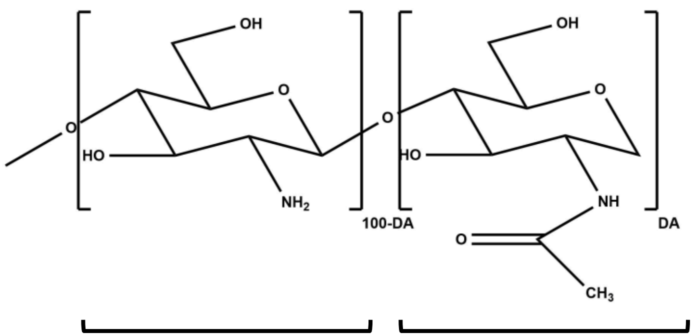


strong Antibacterial activity

Tavares, TD, Antunes, JC *et al.*, *Antibiotics* **2020**, *9*(6), 314

Chitosan (CS) and Poly (vinyl alcohol) (PVA)

CS

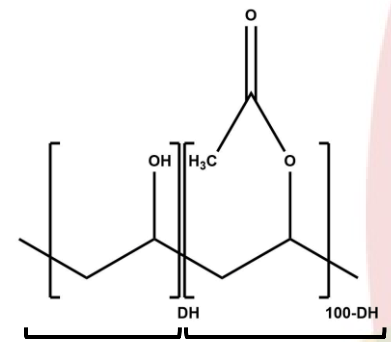


D-glucosamine N-acetyl-D-glucosamine

Natural and crystalline polymer
Biocompatible and biodegradable
Film-forming
High viscosity
Antibacterial and antifungal properties
Ability to absorb exudates

Food and Drug Administration (FDA)-approved as a wound dressing material (topical intended use)

PVA



Poly (vinyl alcohol) Poly (vinyl acetate)

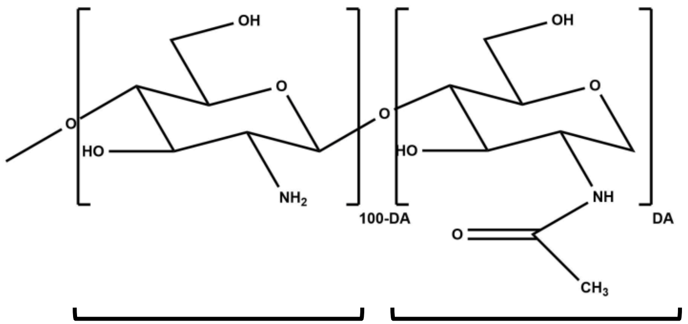
Synthetic and semi-crystalline polymer
Biocompatible and biodegradable
Film-forming
Good mechanical properties: flexibility and swelling capability in aqueous environments
Water-soluble

Multiple FDA-approved medical uses, in the form of transdermal patches, jellies, oral tablets, ophthalmic preparations, intradermal patches and sutures, among others

Production of CS/PVA films

CS

Antimicrobial properties

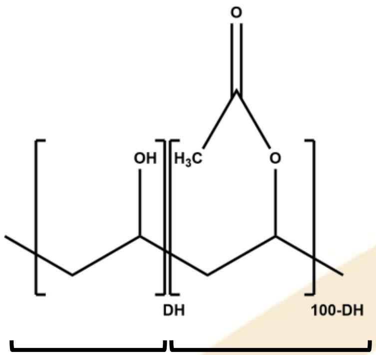


D-glucosamine N-acetyl-D-glucosamine

Blend

PVA

Flexibility and hydrophilicity



Poly (vinyl alcohol) Poly (vinyl acetate)

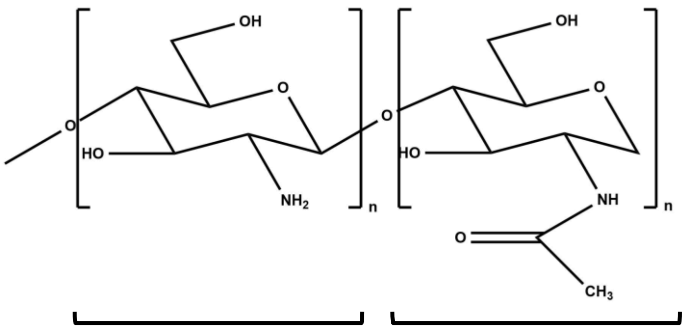
- good capacity to form intermolecular hydrogen bonds
- readily forms hydrogen bonds due to a large number of hydroxyl groups

✓ Increase hydrophilicity, improve mechanical properties

✓ Improve stability in aqueous environments

Production of CS/PVA films

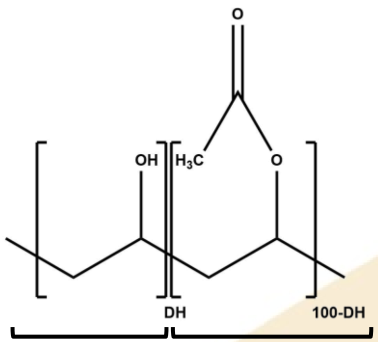
CS



D-glucosamine N-acetyl-D-glucosamine

Blend

PVA



Poly (vinyl alcohol) Poly (vinyl acetate)

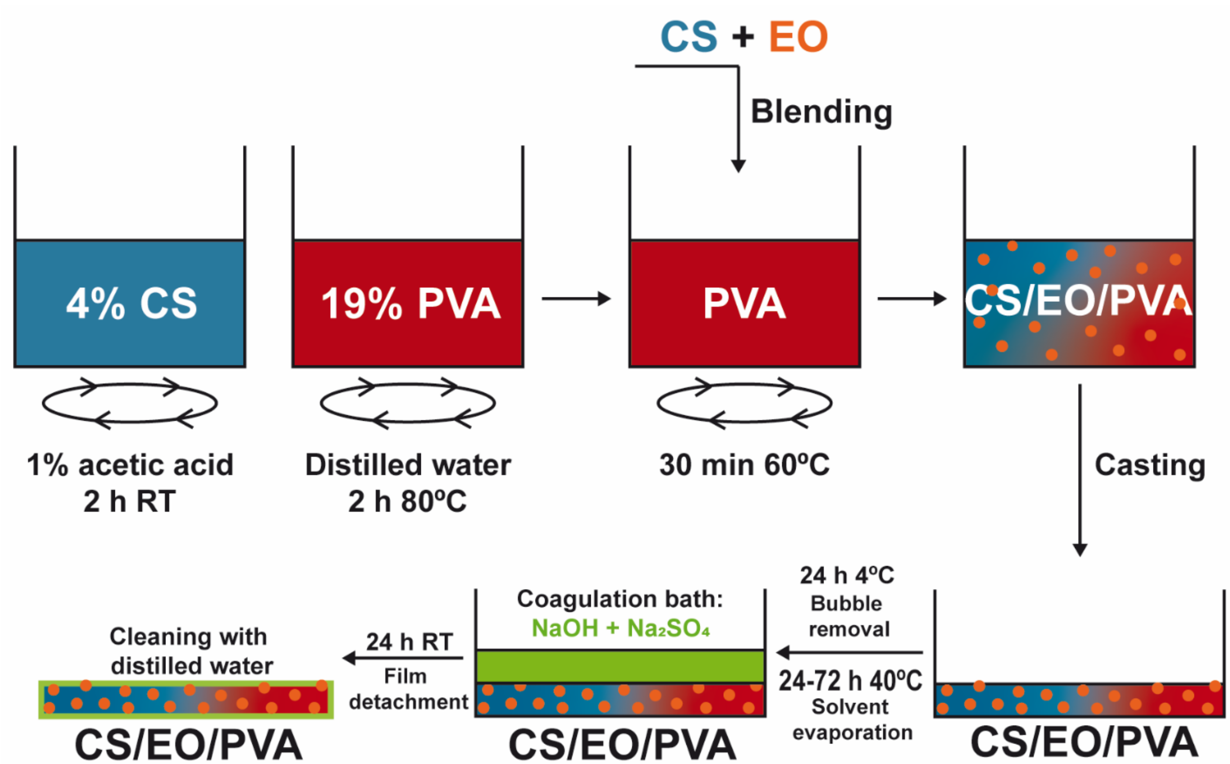
Main Applications:

Food packaging, controlled release of biomolecules, wound dressing, tissue engineering, membrane bioreactors, pervaporation, reverse osmosis, dye removal, fuel cells

Production of CS/EO/PVA films

Solvent Casting + Phase Inversion

CS: 100-300 kDa and 9.6±1.4% DA **PVA:** 72 kDa and 88% DH



JC Antunes *et al.*, Pharmaceutics (2021) doi:10.3390/pharmaceutics13020195

Production of CS/EO/PVA films

Solvent Casting + Phase Inversion

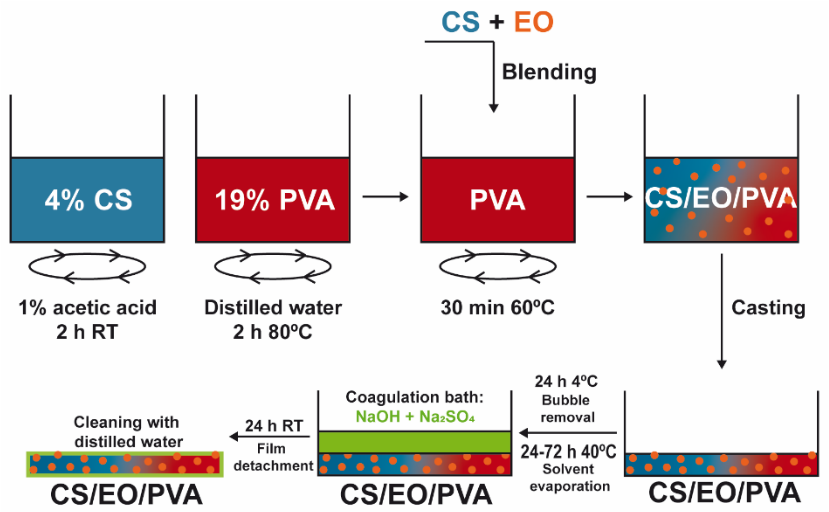
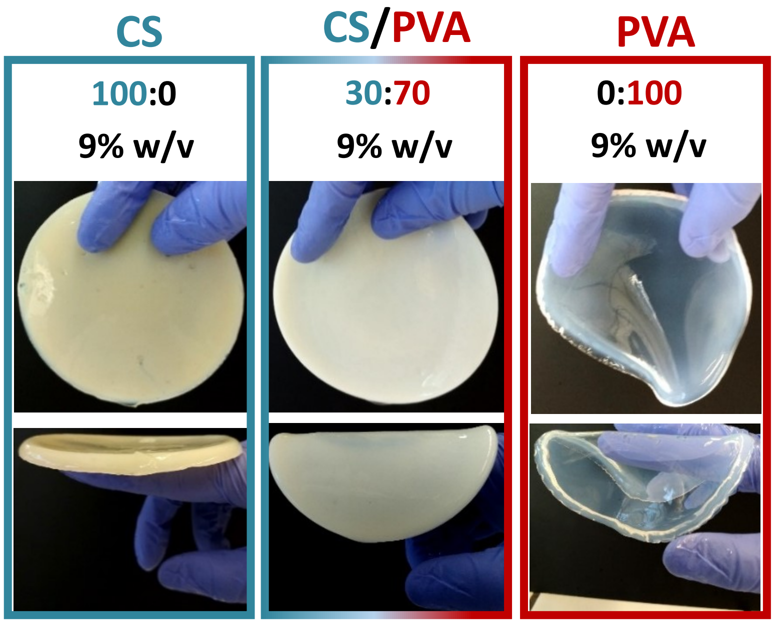


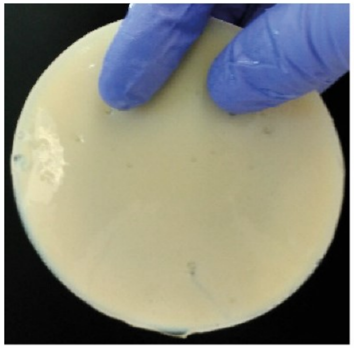
Table 2. Data required to build tested CS/EO/PVA blended films, specifically EO loading amount (in µL), mass (g), and volume (mL) of polymer solutions for each case, total mass percent (%*w*/*v*), total volume (mL), and selected CS/PVA mass ratios.

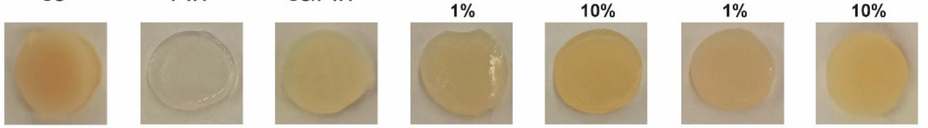
	EO		CS Solution		PVA Solution		Total % *w*/*v*	V_{Total} (mL)	CS/PVA Mass Ratios
	m (mg)	V (µL)	m_{CS} (g)	V (mL)	m_{PVA} (g)	V (mL)			
CS	-	-	3.51	39	-	-	9	39	100/0
PVA	-	-	-	-	3.51	39	9	39	0/100
CS/PVA	-	-	1.05	26	2.46	13	9	39	30/70
CS/PVA/CLO 1%	35.1	39.2	1.05	26	2.46	13	9	39	30/70
CS/PVA/CLO 10%	351.0	392.0	1.05	26	2.46	13	9	39	30/70
CS/PVA/CO 1%	35.1	33.2	1.05	26	2.46	13	9	39	30/70
CS/PVA/CO 10%	351.0	332.0	1.05	26	2.46	13	9	39	30/70

4% CS and 19% PVA solutions were used.

Characterization of CS/EO/PVA films





	CS	PVA	CS/PVA	CS/PVA/CLO 1%	CS/PVA/CLO 10%	CS/PVA/CO 1%	CS/PVA/CO 10%
							
Thickness (mm)	1.73 ± 0.11**	0.47 ± 0.06	0.72 ± 0.02	0.95 ± 0.03	1.31 ± 0.07*	0.83 ± 0.04	1.08 ± 0.14
DS (%)	87.45 ± 6.04	72.01 ± 6.68	85.22 ± 2.93	85.33 ± 2.70	92.49 ± 0.25*	86.73 ± 2.10	90.96 ± 0.96

Statistical significance (**p < 0.005) found through the Kruskal-Wallis test, followed by the Dunn's multiple comparisons test, to compare each unpaired group (n=4).

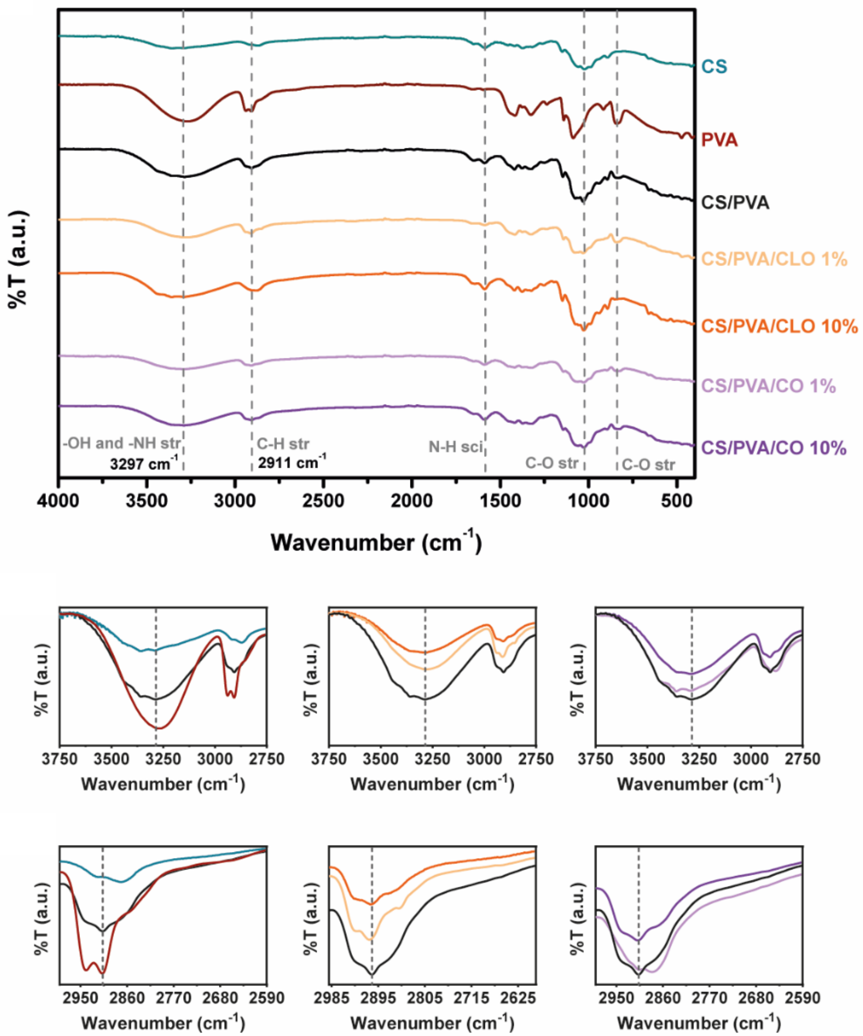
Hydrophobic **EO** loading —resulted in→ increased film thickness up to 182 (10% CLO) and overall water retention capacity

suggesting ↓

Polymer chain rearrangements and EO entrapment inside the matrix

Characterization of CS/EO/PVA films

CS/EO/PVA film:



Peaks of both polymers are present
No new peaks are formed

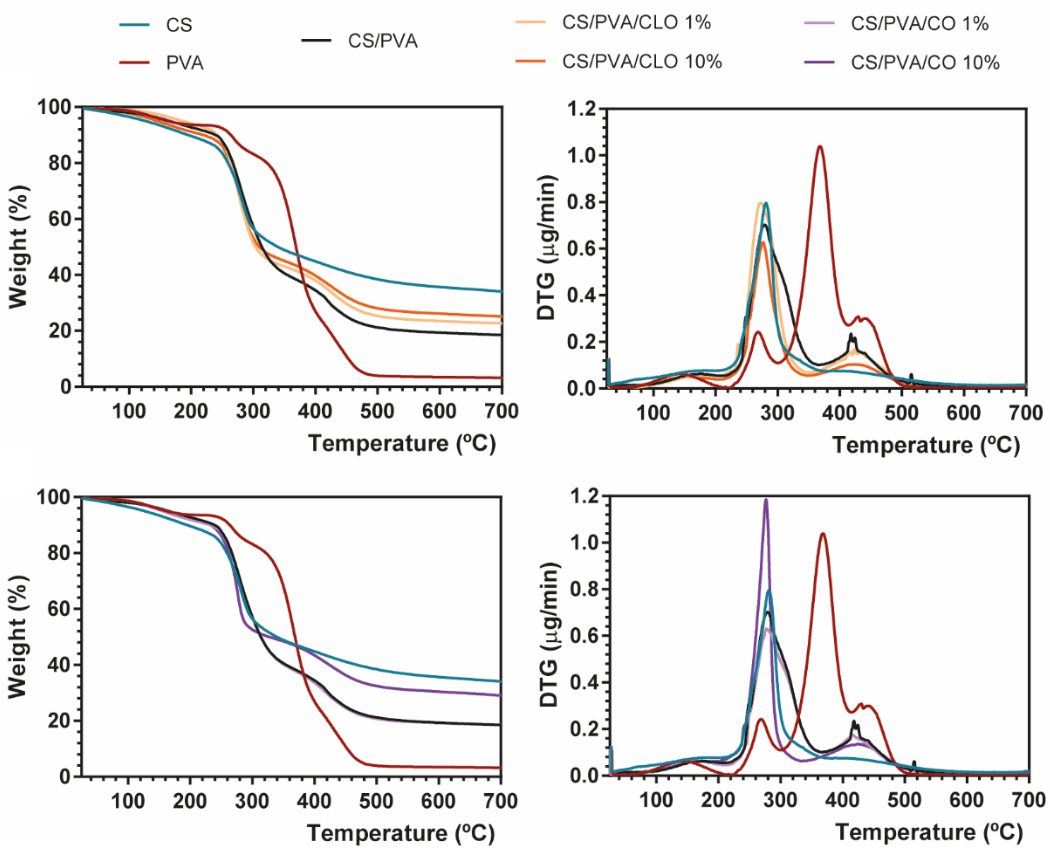
suggesting

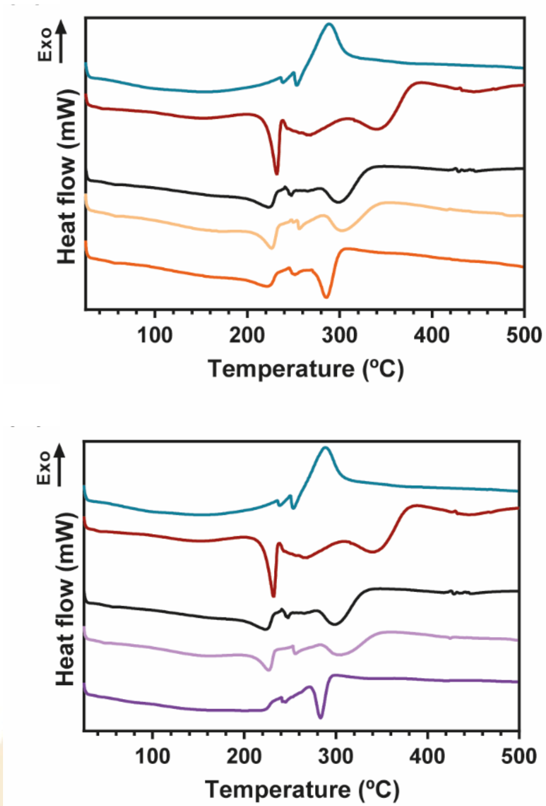
Polymers blend
Hydrogen bond formation

suggesting

Commitment of free -OH groups with increasing EO amount is noticeable with both EOs

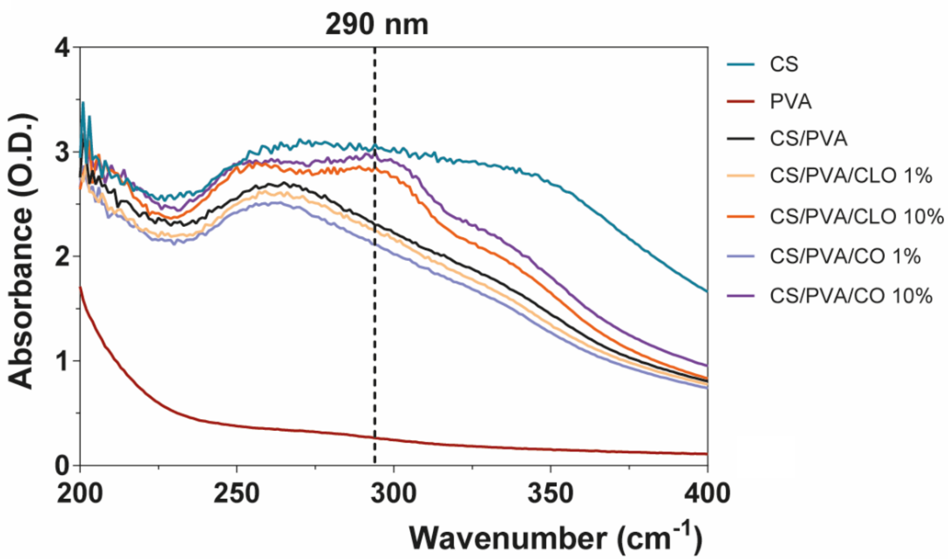
Characterization of CS/EO/PVA films

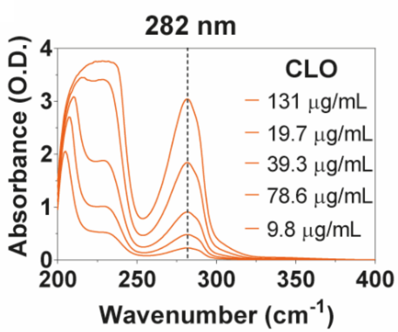


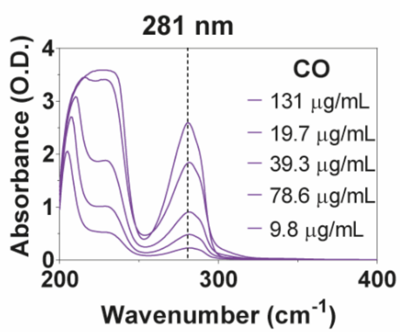


Film's thermal-induced behaviour → **reinforcing** → Polymer blending and EO entrapment

Characterization of CS/EO/PVA films

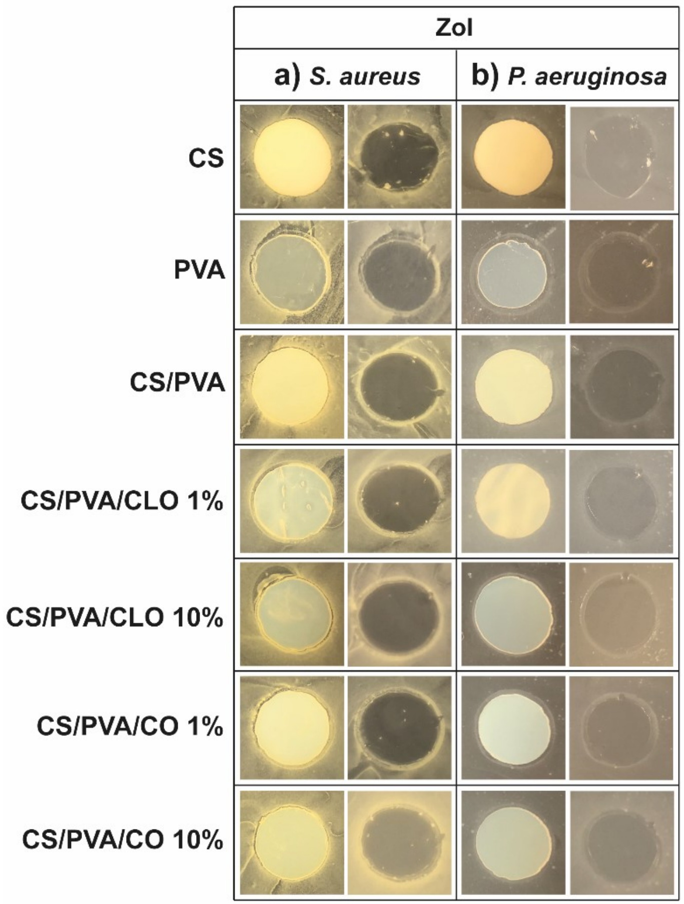






	EO mass (μg)
CS/PVA/CLO 1%	**0.050**
CS/PVA/CLO 10%	**0.265**
CS/PVA/CO 1%	**0.038**
CS/PVA/CO 10%	**0.202**

Antibacterial testing



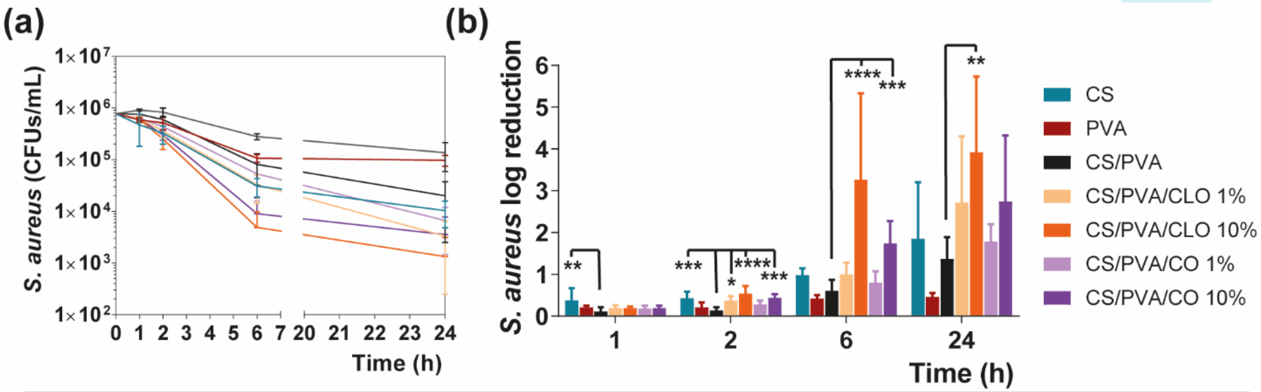
For each bacterium, left images depict films at their original location at the beginning of the assay, along with the bacteria that grew over the incubation period; while on the right, cultured films were carefully removed from the agar so that contact-kill could be visualized.

24h of incubation

↓

Slight antibacterial features

Antibacterial testing



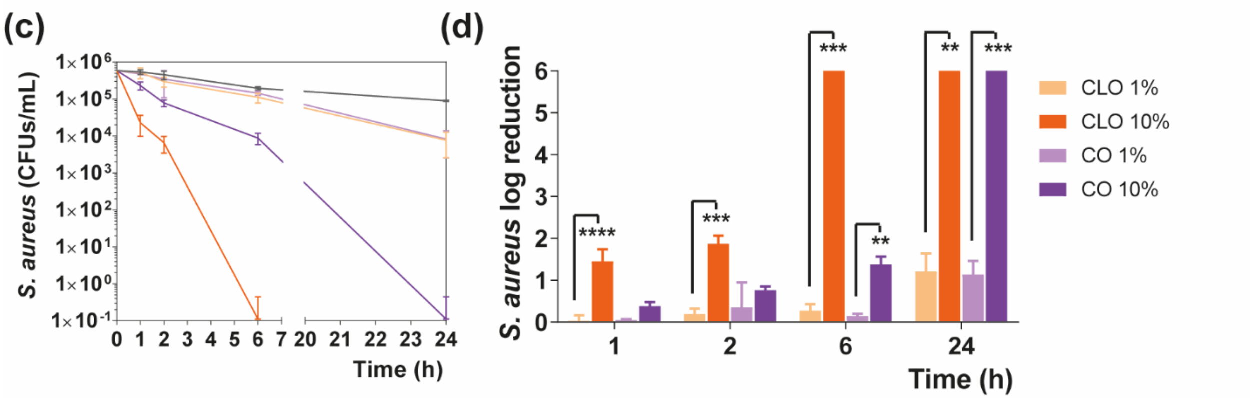
	CS/PVACLO 1%				CS/PVA/CLO 10%				CS/PVA/CO 1%				CS/PVA/CO 10%			
	1	2	6	24	1	2	6	24	1	2	6	24	1	2	6	24
1				****			***					***			**	****
2												*				
6																
24																

CS/CLO 10%/PVA film:

the most effective,
right after 6h with 10% EO

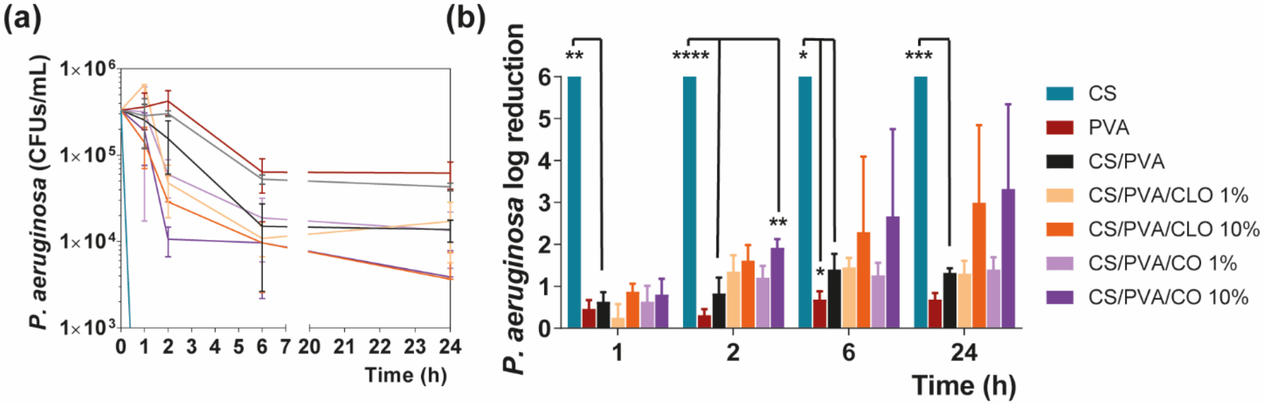
CS film:

quickest AM action
within 1h of incubation



	CLO 1%				CLO 10%				CO 1%				CO 10%			
	1	2	6	24	1	2	6	24	1	2	6	24	1	2	6	24
1				****			****	**				***			**	****
2				**			***					**				**
6																
24																

Antibacterial testing

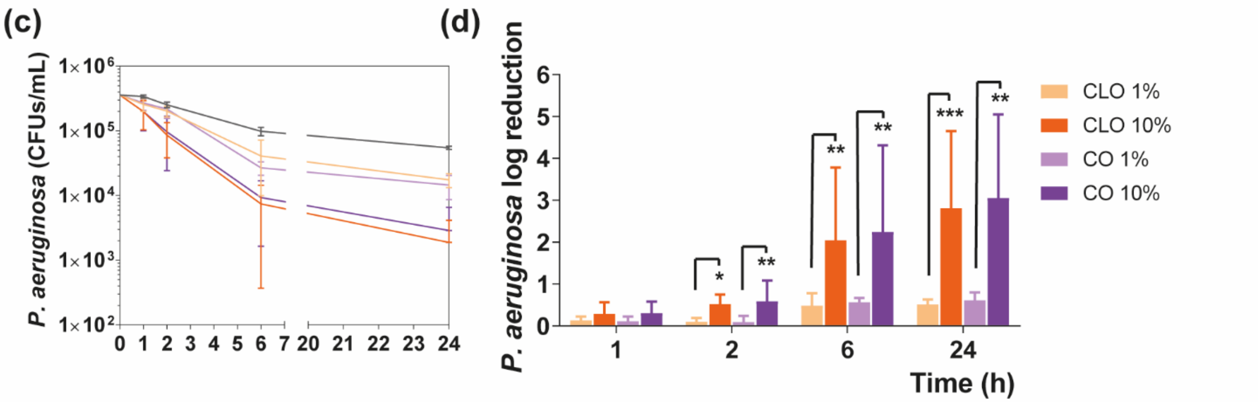


CS/PVA/CLO 1%	1	2	6	24
1			*	
2				
6				
24				

CS/PVA/CLO 10%	1	2	6	24
1				***
2				
6				
24				

CS/PVA/CO 1%	1	2	6	24
1				
2				
6				
24				

CS/PVA/CO 10%	1	2	6	24
1		***		**
2				
6				
24				



CLO 1%	1	2	6	24
1				*
2			*	**
6				
24				

CLO 10%	1	2	6	24
1			**	****
2			*	**
6				
24				

CO 1%	1	2	6	24
1			**	**
2			***	***
6				
24				

CO 10%	1	2	6	24
1			**	***
2				*
6				
24				

CS/EO/PVA film:

10% CO led to a clear bactericidal trend after 2h of contact

CS film:

Complete bacterial elimination in 1h, effect that endured until tested 24h

Conclusions and Future Work

- ✓ CS/PVA blended films were successfully built;
- ✓ CS and both EOS, the CLO and CO, show antibacterial activity against *S. aureus* and *P. aeruginosa;*
- ✓ The EOs were successfully incorporated in the CS/PVA films at 1 and 10%wt;
- ✓ CLO-loaded CS/PVA films showed evidently bactericidal effects right after 2h of direct contact with the bacteria, being significantly more efficient than unloaded films until the tested 24h.
- ✓ Films with 100% CS were particularly more effective than 10% EOO-loaded films against *P. aeruginosa*, by completely eradicating it during the first hour of incubation.

Future work will be directed towards a balance between AM action of CS and its mechanical hindrance after processing, together with the combination with the EOs to an intensified antimicrobial profile against both bacteria.

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