Universidade do Minho
Escola de Engenharia

CENTRO DE CIÊNCIA E TECNOLOGIA TÊXTIL

Antibacterial activity of marine-derived chitosan and plant-derived cajeput oil as loaded blended films in *Staphylococcus aureus* and *Pseudomonas aeruginosa*-enriched settings

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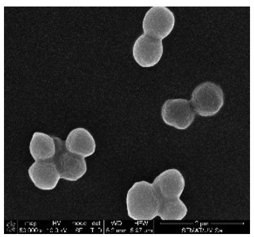
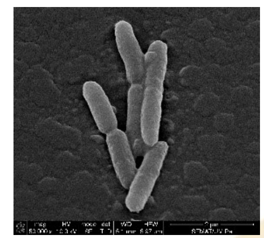
Infected wounds

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

↓

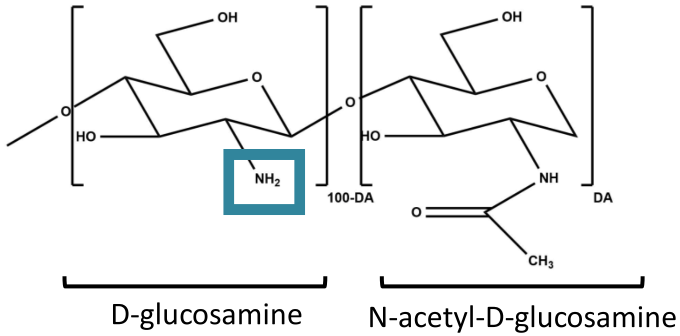
serious concerns about DFU therapeutic strategies

↓

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 CJO

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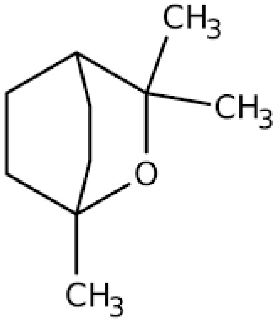
Essential oils (EOs):

- ✓ 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 1,8-Cineole

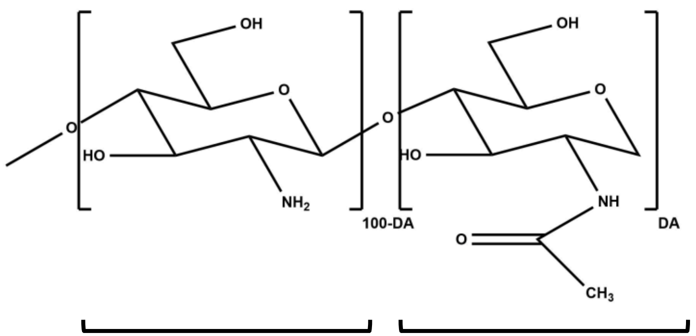


strong antibacterial activity

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

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

CS

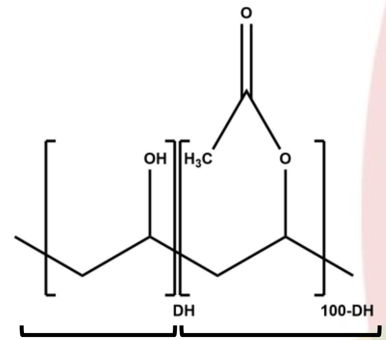


D-glucosamine N-acetyl-D-glucosamine

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)

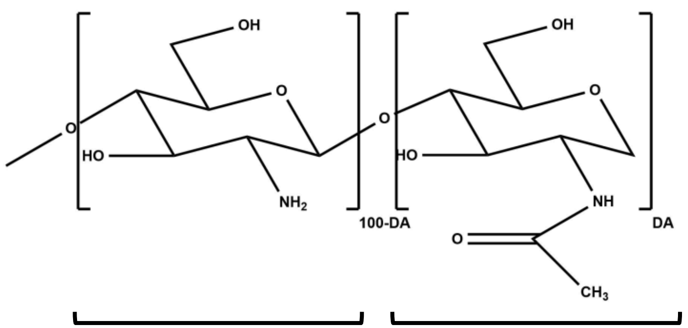
Synthetic and semi-crystalline polymer
Biocompatible and biodegradable
Film-forming
Good mechanical properties: flexibility and swelling capability in aqueous environments
Water-soluble

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/CJO/PVA films

CS

Antimicrobial properties

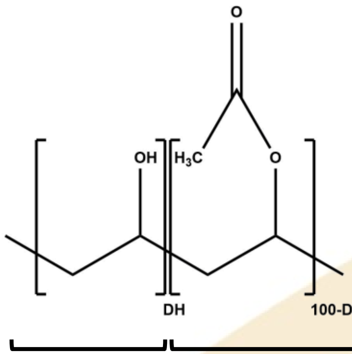


D-glucosamine N-acetyl-D-glucosamine

Blend

PVA

Flexibility and hydrophilicity



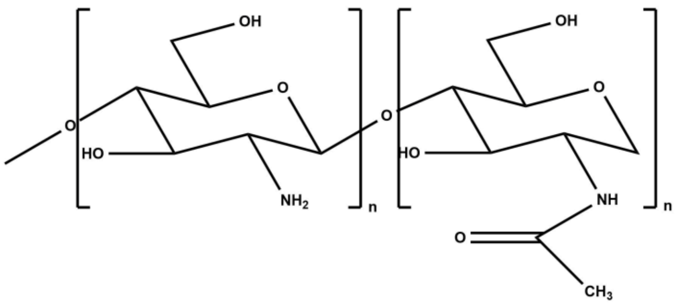
Poly (vinyl alcohol) Poly (vinyl acetate)

- 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/CJO/PVA films

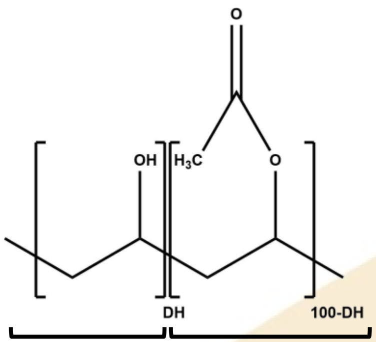
CS



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/CJO/PVA films

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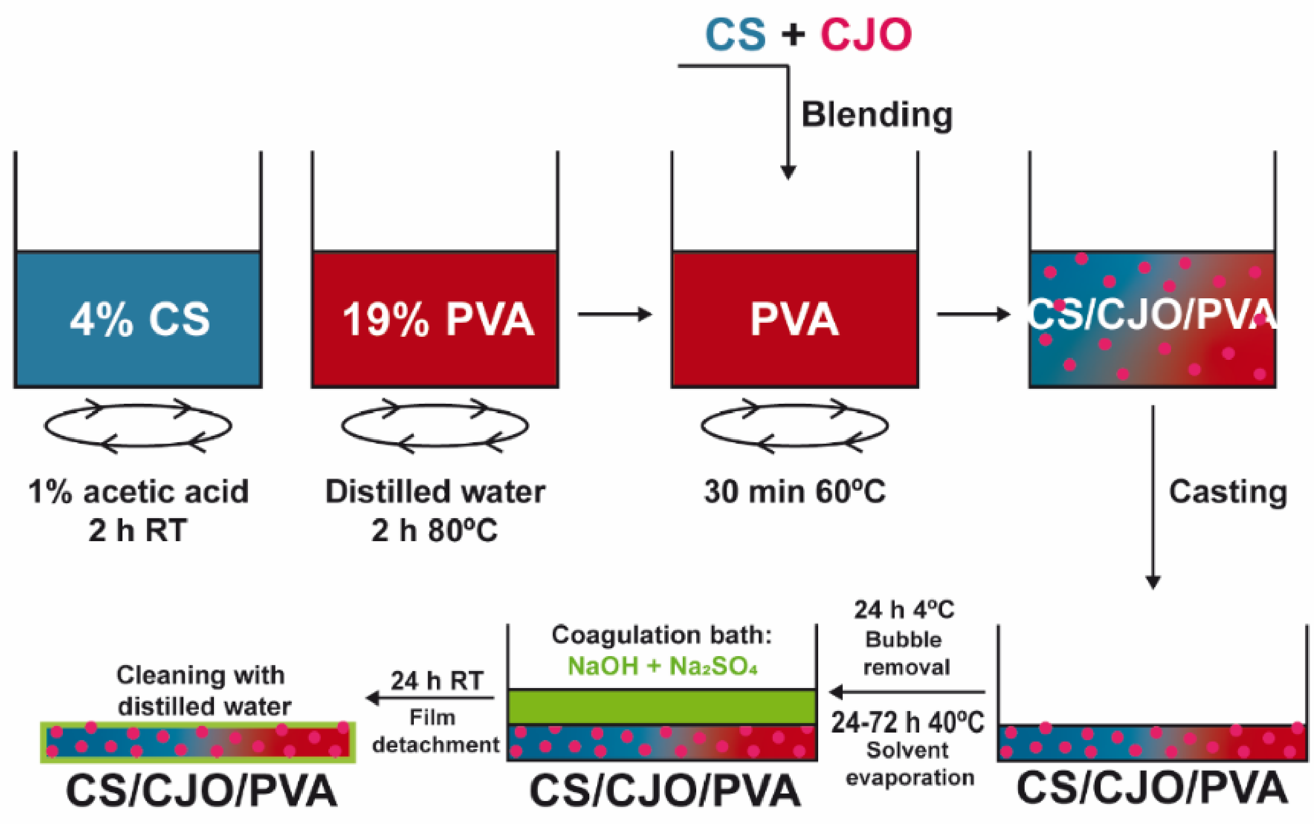
Solvent Casting + Phase Inversion

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



adapted from HP Felgueiras *et al.*, J Appl Polym Sci (2019) doi: 10.1002/app.43626
J. Appl. Polym. Sci. 2018, doi: 10.1002/APP.46188

Production of CS/CJO/PVA films

Solvent Casting + Phase Inversion

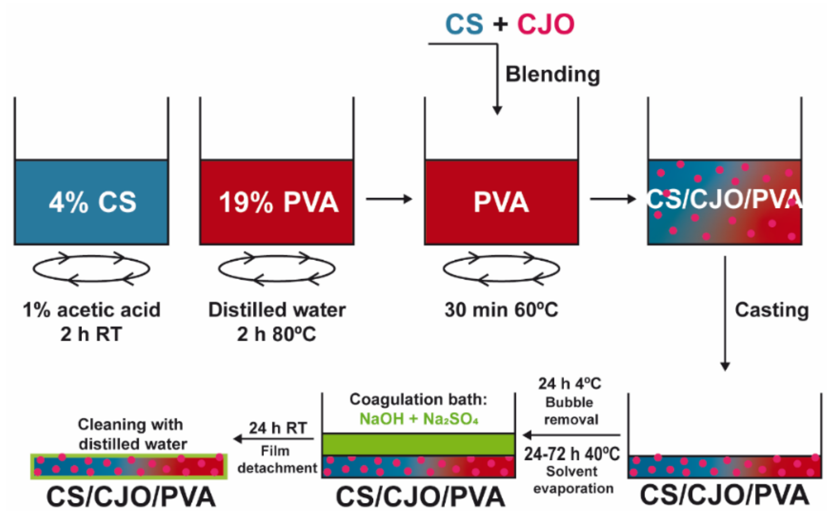
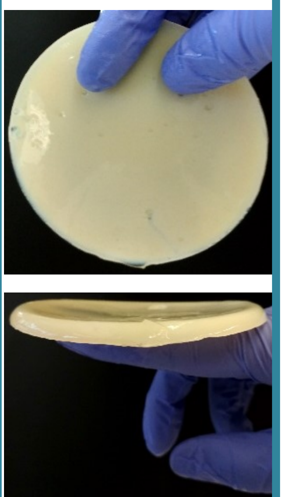
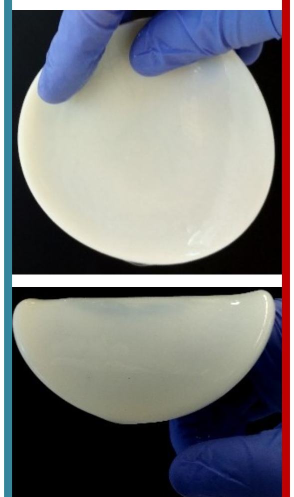
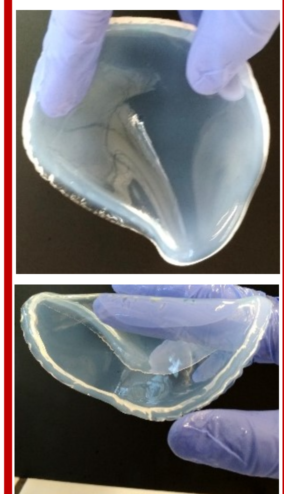
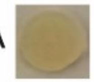


Table. Practical numbers required to build tested CS/CJO/PVA blended films, specifically CJO loading amount (in µL), mass (g) and volume (mL) of polymer solutions for each case, total mass percent (%m/V), total volume (mL) and selected CS/PVA mass ratios.

	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	-	-			100/0
PVA	-	-	-	-	3.51	39			0/100
CS/PVA	-	-					9%	39	
CS/PVA/CJO 1%	35.1	39.2	1.053	26	2.457	13			30/70
CS/PVA/CJO 10%	351	392							

Characterization of CS/CJOPVA films

CS	CS/PVA	PVA
100:0	30:70	0:100
9% w/v	9% w/v	9% w/v
		

		Thickness (mm)	Degree of Swelling (%)	Porosity
CS		1.73 ± 0.11**	87.45 ± 6.04	87.44 ± 3.68
PVA		0.47 ± 0.06	72.01 ± 6.68	76.42 ± 8.91
CS/PVA		0.72 ± 0.02	85.22 ± 2.93	89.52 ± 4.62
CS/PVA/CJO 1%		0.89 ± 0.05	85.87 ± 1.18	90.15 ± 4.34
CS/PVA/CJO 10%		1.14 ± 0.10	88.50 ± 1.74	91.83 ± 5.25

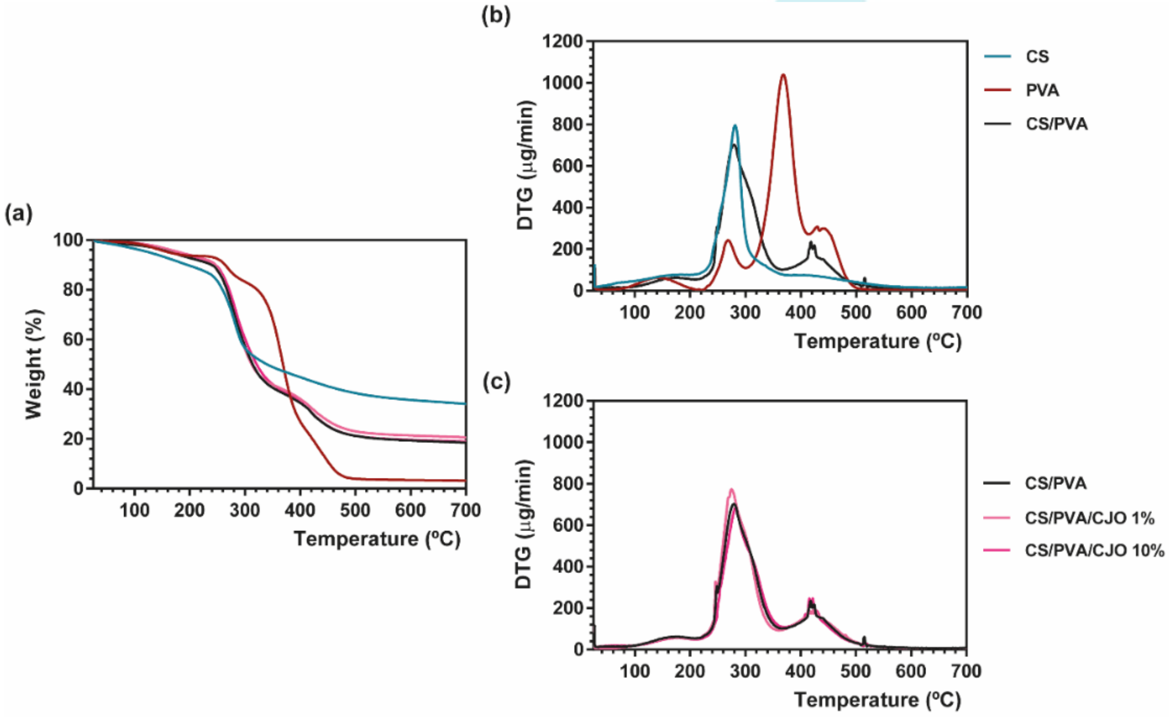
Statistical significance (**$p < 0.005$) found through the Kruskal-Wallis test, followed by the Dunn's multiple comparisons test, to compare each unpaired group (n=4).

Hydrophobic CJO loading **resulted in** → increased film thickness up to 124 (1% CJO) or 158% (10% CJO), overall water retention capacity, and porosity

suggesting ↓

Polymer chain rearrangements and EO entrapment inside the matrix

Characterization of CS/CJO/PVA films



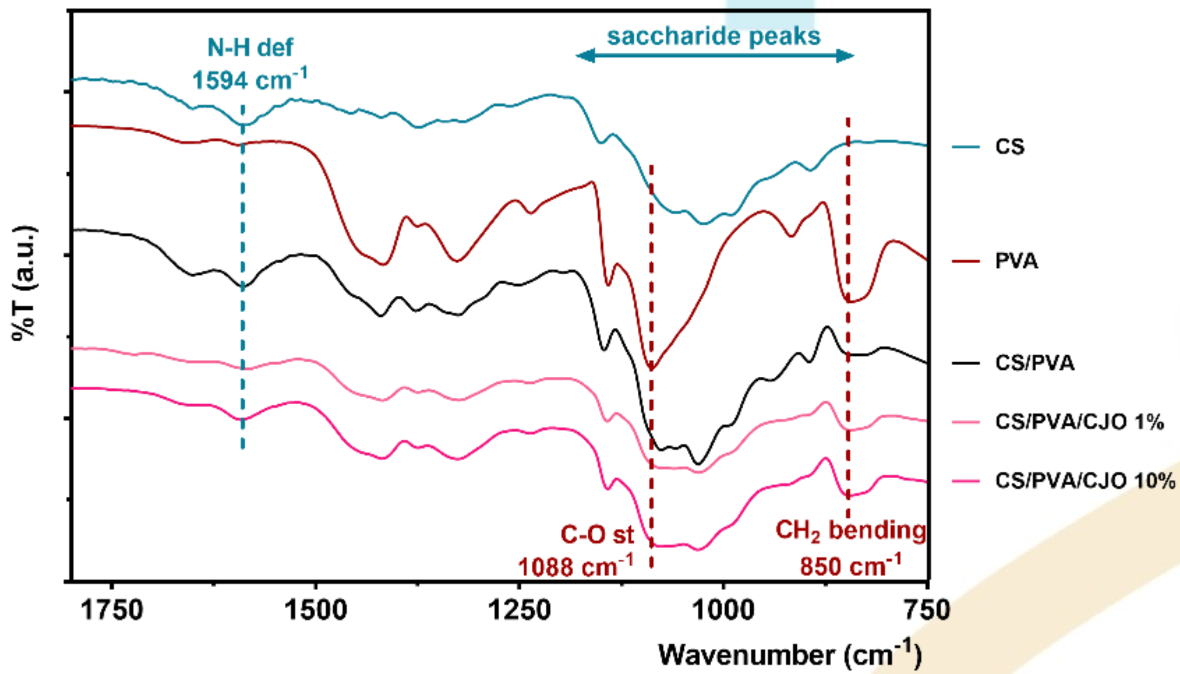
CS/CLO/PVA film:

Similar thermal-induced behaviour than unloaded films
No peaks shifts are detected

suggesting →

Neglectable EO influence on film's thermal properties

Characterization of CS/CJO/PVA films



CS/CLO/PVA film:

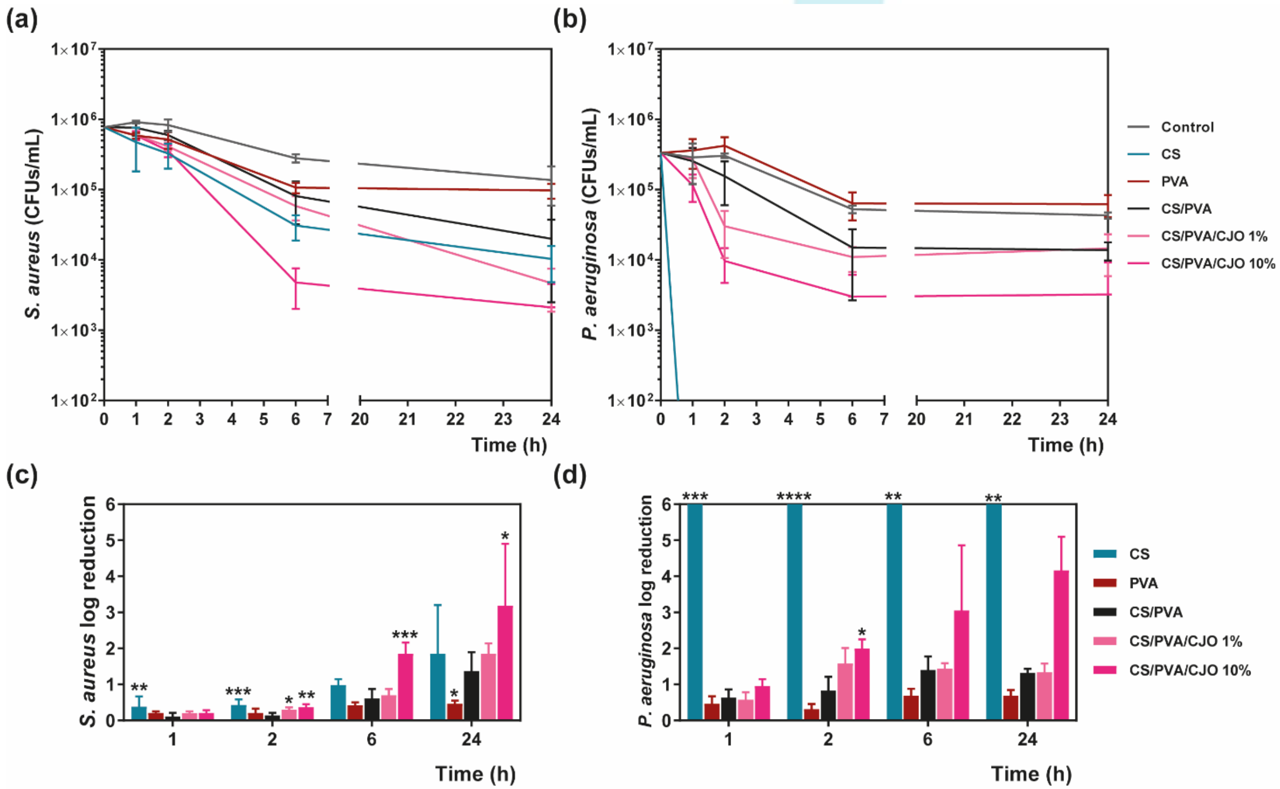
Peaks of both polymers are present
No new peaks are formed

suggesting →

Polymers blend
Hydrogen bond formation

Neglectable EO influence
on film's chemical composition

Antibacterial testing



CS/CLO/PVA film:

S. aureus:

the most effective after 6h with 10% EO

P. aeruginosa:

10% CJO led to an increasingly bactericidal trend, clear after 2h of contact

CS film:

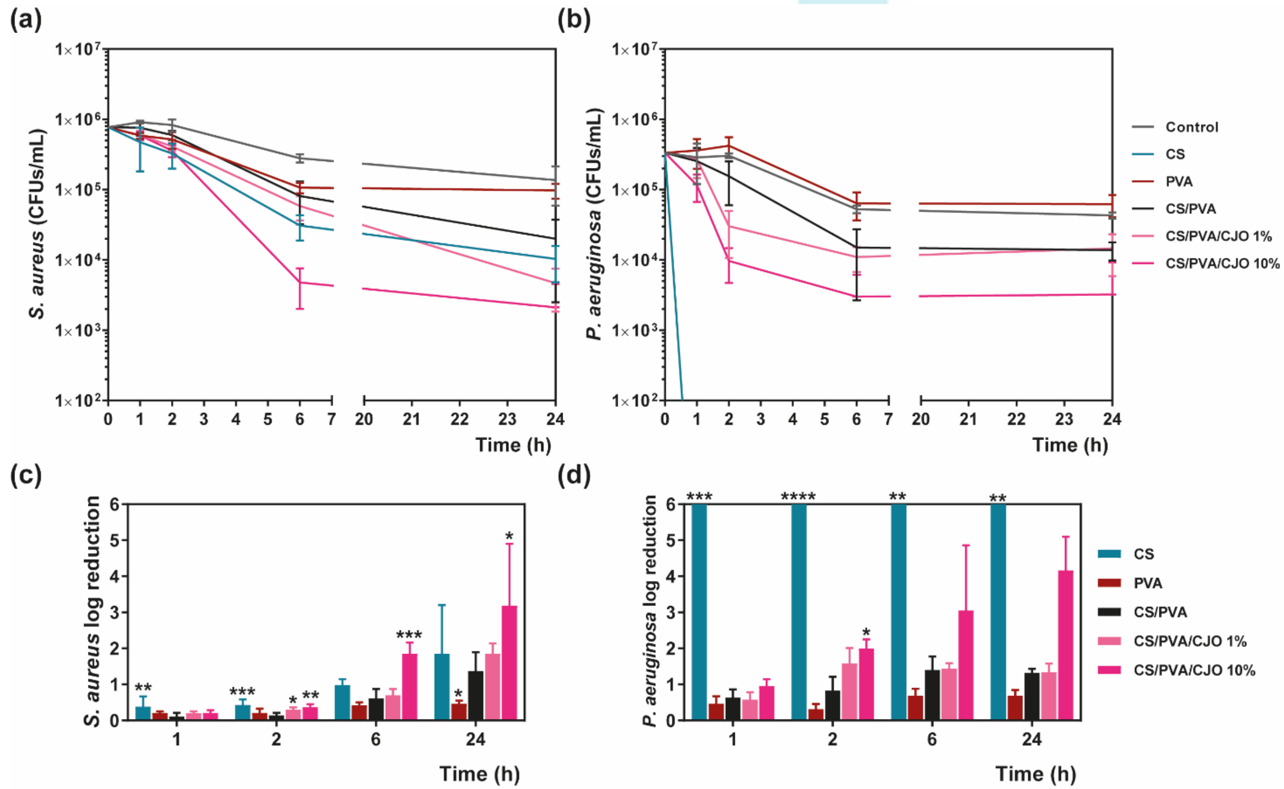
S. aureus:

quickest AM action within 1h of incubation

P. aeruginosa:

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

Antibacterial testing



CS/CLO/PVA film:

CS film:

Reinforced antibacterial action when CJO is added to the CS-based films

10% CJO led to an increasingly bactericidal trend, clear after 2h of contact

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

Conclusions and Future Work

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- ✓ CS/PVA blended films were successfully built;
- ✓ Both CS and CJO show antibacterial activity against *S. aureus* and *P. aeruginosa;*

Felgueiras, HP *et al., Biomolecules* **2020**, *10*(8), 1

- ✓ CJO was successfully incorporated in the CS/PVA films at 1 and 10%wt;
- ✓ CJO-loaded CS/PVA films showed evidently bactericidal effects following 2h of direct contact with the bacteria, being significantly more efficient than unloaded films.
- ✓ Films with 100% CS were particularly more effective than 10% CJO-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 CJO to an intensified antimicrobial profile against both bacteria.

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PEPTEX Project:

Electrospun polymeric wound dressings functionalized with Tiger 17 for an improved antimicrobial protection and faster tissue regeneration in pressure ulcers

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