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Combinatory action of chitosan-based blended films and loaded cajeput oil against *Staphylococcus aureus* and *Pseudomonas aeruginosa*-mediated infections

Chaired by **PROF. DR. ANTONIO PIZZI** and **PROF. DR. FRANK WIESBROCK**

polymers MDPI

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2C2T CENTRO DE CIÊNCIA E TECNOLOGIA TÊXTIL

Abstract: Chronic wounds (CW) have numerous entry ways for pathogen invasion and prosperity, damaging host tissue and hindering tissue remodeling. Essential oils exert quick and efficient antimicrobial (AM) action, unlikely to induce bacterial resistance. Cajeput oil (CJO) has strong AM properties, namely against Staphylococcus aureus and Pseudomonas aeruginosa. Chitosan (CS) is a natural and biodegradable cationic polysaccharide, also widely known for its AM features. CS and poly(vinyl alcohol) (PVA) films were prepared (ratio 30/70; 9%wt) by solvent casting and phase inversion method. Film's thermal stability and chemical composition data reinforce polymer blending. Films were supplemented with 1 and 10wt% of CJO in relation to total polymeric mass. Loaded films were 23 and 57% thicker, respectively, than the unloaded films. Degree of swelling and porosity also increased, particularly with 10wt% CJO. AM testing revealed that CS films alone were effective against both bacteria, eradicating all P. aeruginosa within the hour (***$p<0.001$). Still, loaded CS/PVA films showed improved AM traits, being significantly more efficient than unloaded films right after 2h of contact. This study is a first proof of concept that CJO can be dispersed into CS/PVA films and show bactericidal effects, particularly against P. aeruginosa, this way opening new avenues for CW therapeutics.

Keywords: bactericidal, marine-derived polymers, natural bioactive agents, drug delivery systems, blended films.

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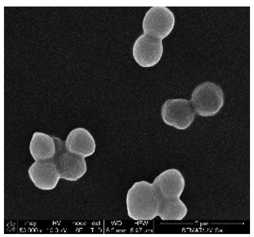
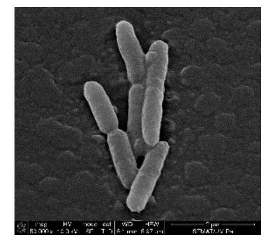
Infected Wounds

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

↓

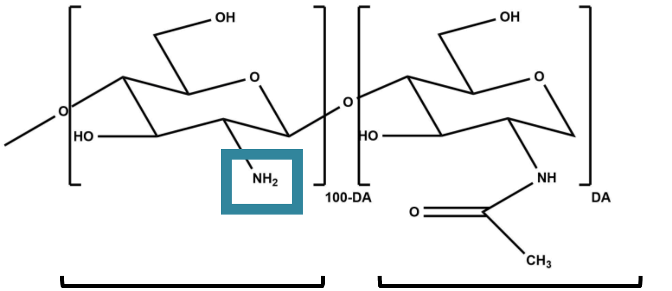
serious concerns about DFU therapeutic strategies

↓

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



D-glucosamine N-acetyl-D-glucosamine

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 permeability (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, M_W, DA, viscosity, solvent and concentration)

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

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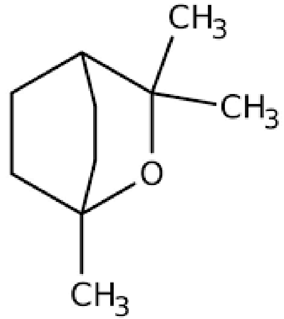
Antibacterial CJO

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 antimicrobial activity

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

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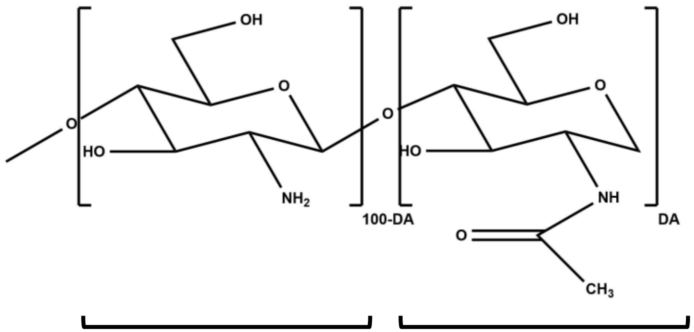
Chitosan (CS) and Poly (vinyl alcohol) (PVA)

CS

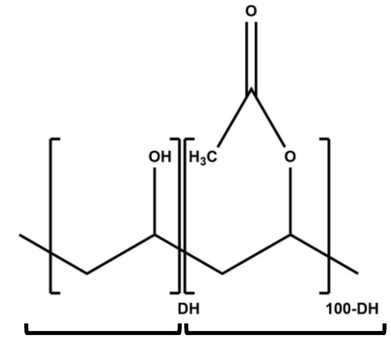


D-glucosamine N-acetyl-D-glucosamine

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)

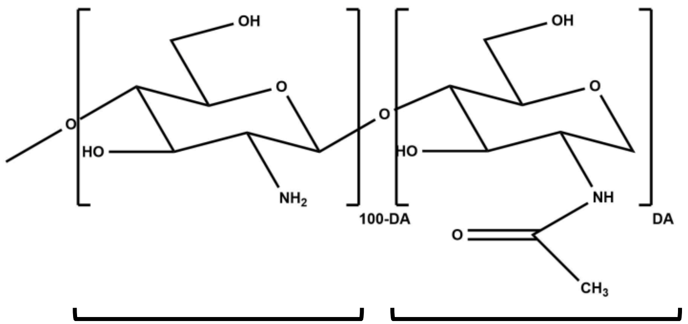
Synthetic and semi-crystalline polymer
Biocompatible and biodegradable
Film-forming
Good mechanical properties: flexibility and swelling capability in aqueous environments
Water-soluble

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

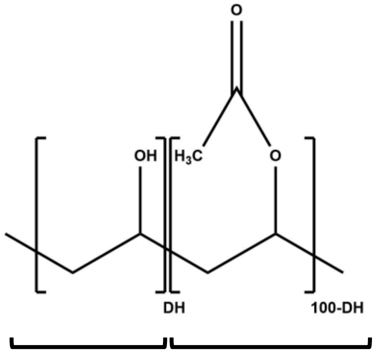


D-glucosamine N-acetyl-D-glucosamine

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

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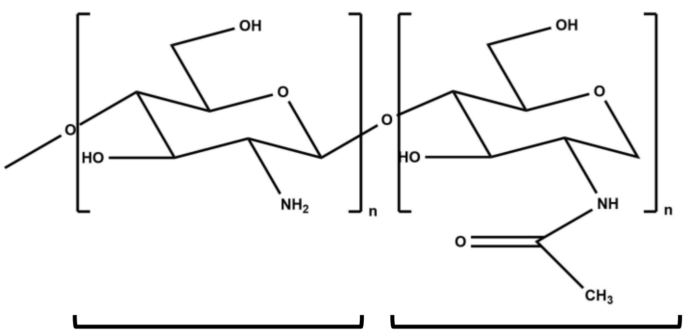
Production of CS/CJO/PVA films

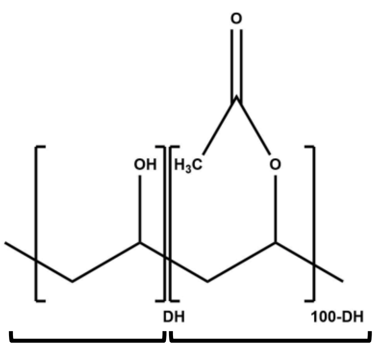
CS



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

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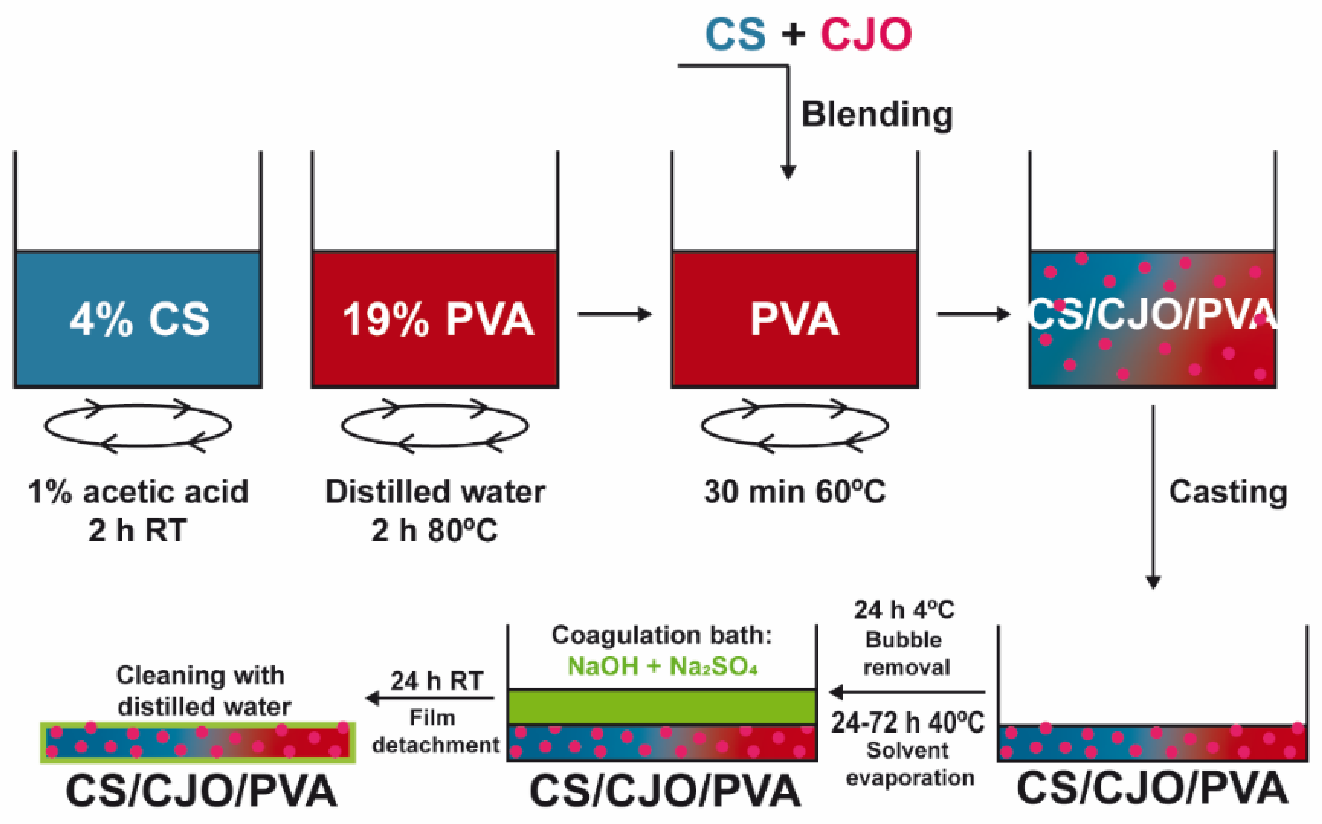
Production of CS/CJO/PVA films

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.48626
J. Appl. Polym. Sci. 2018, doi: 10.1002/APP.46188

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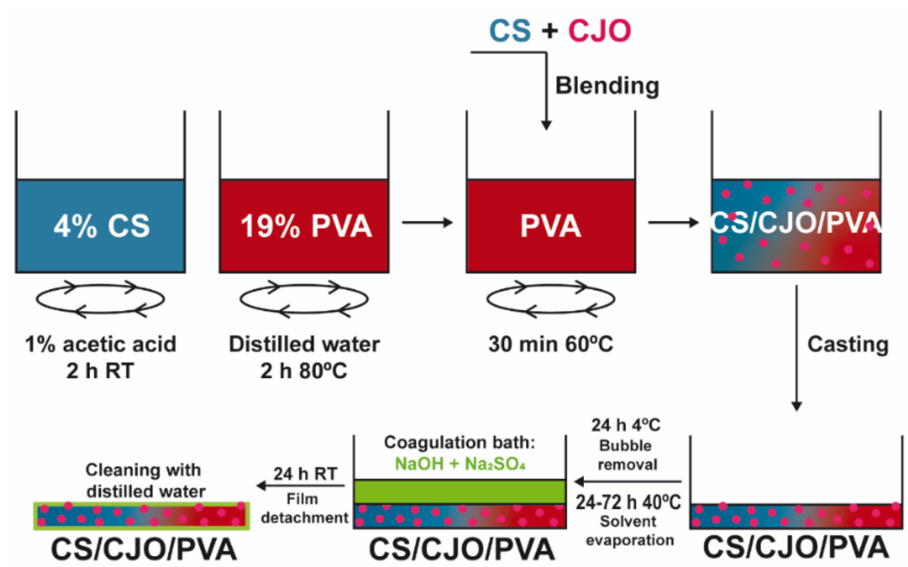
Production of CS/CJO/PVA films

Solvent Casting + Phase Inversion

	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							



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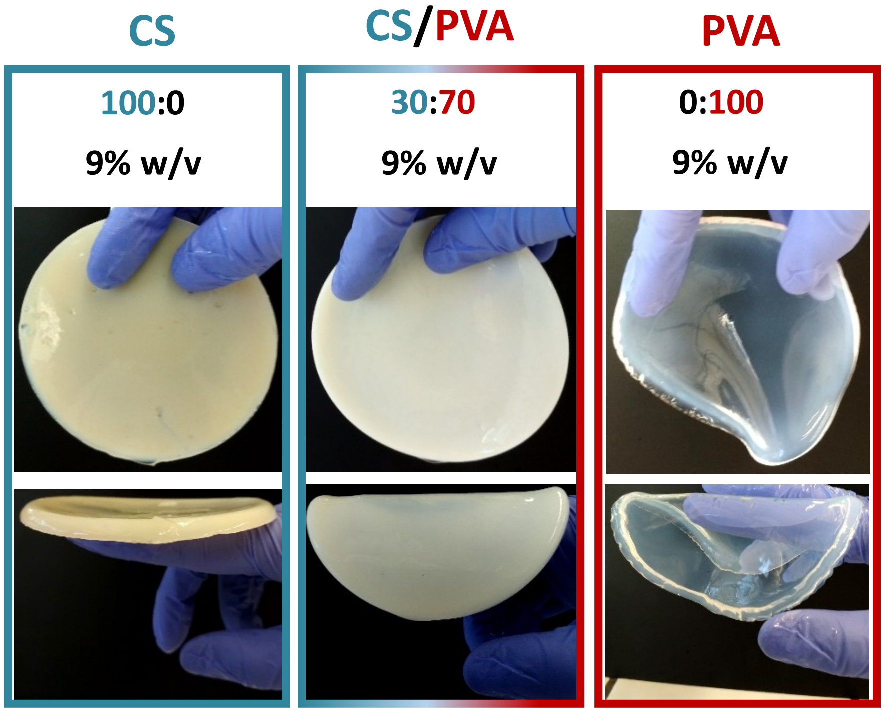
Characterization of CS/CJO/PVA films



	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

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

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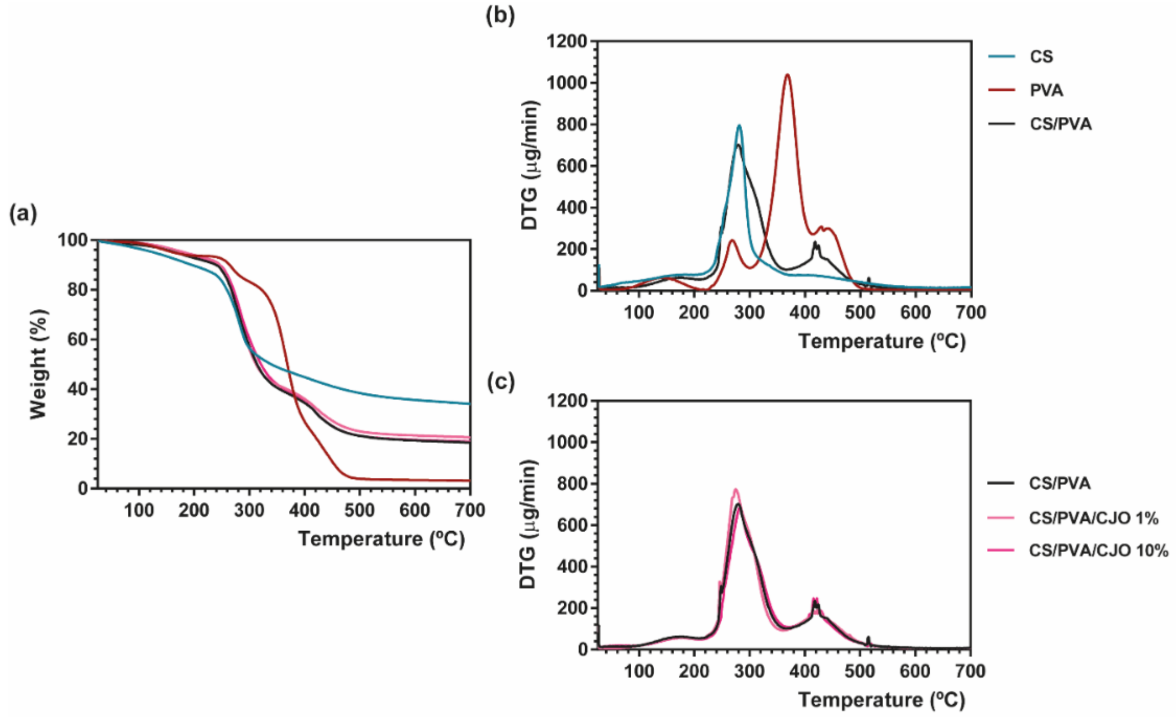
Characterization of CS/CJO/PVA films



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

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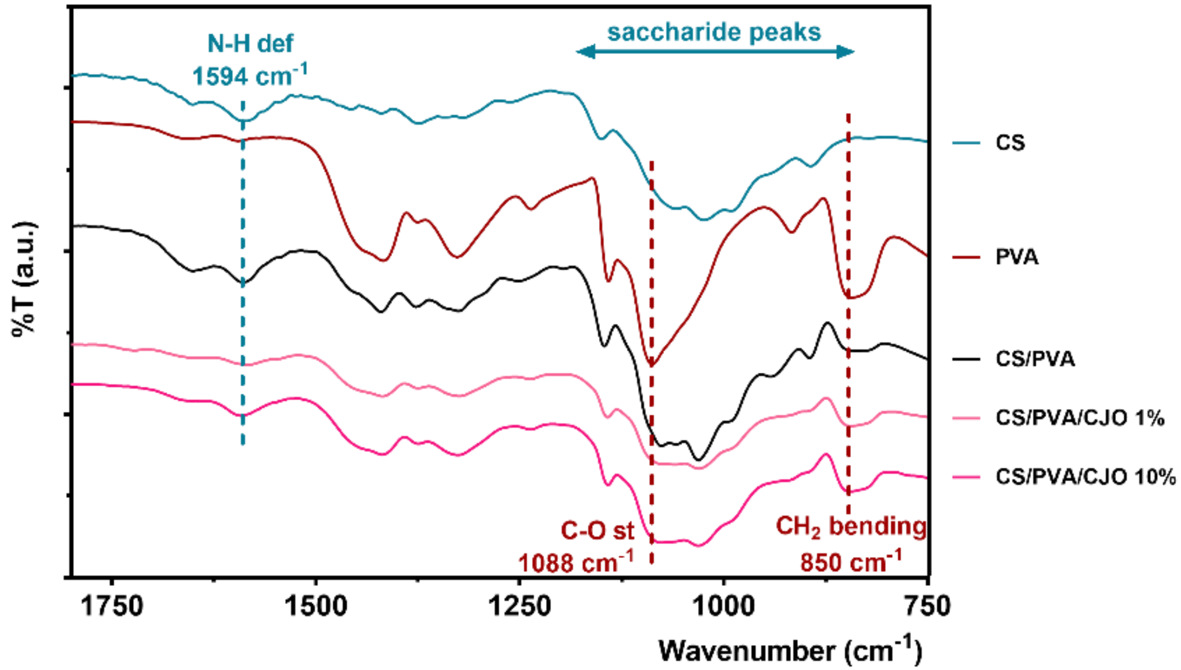
Characterization of CS/CJO/PVA films



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

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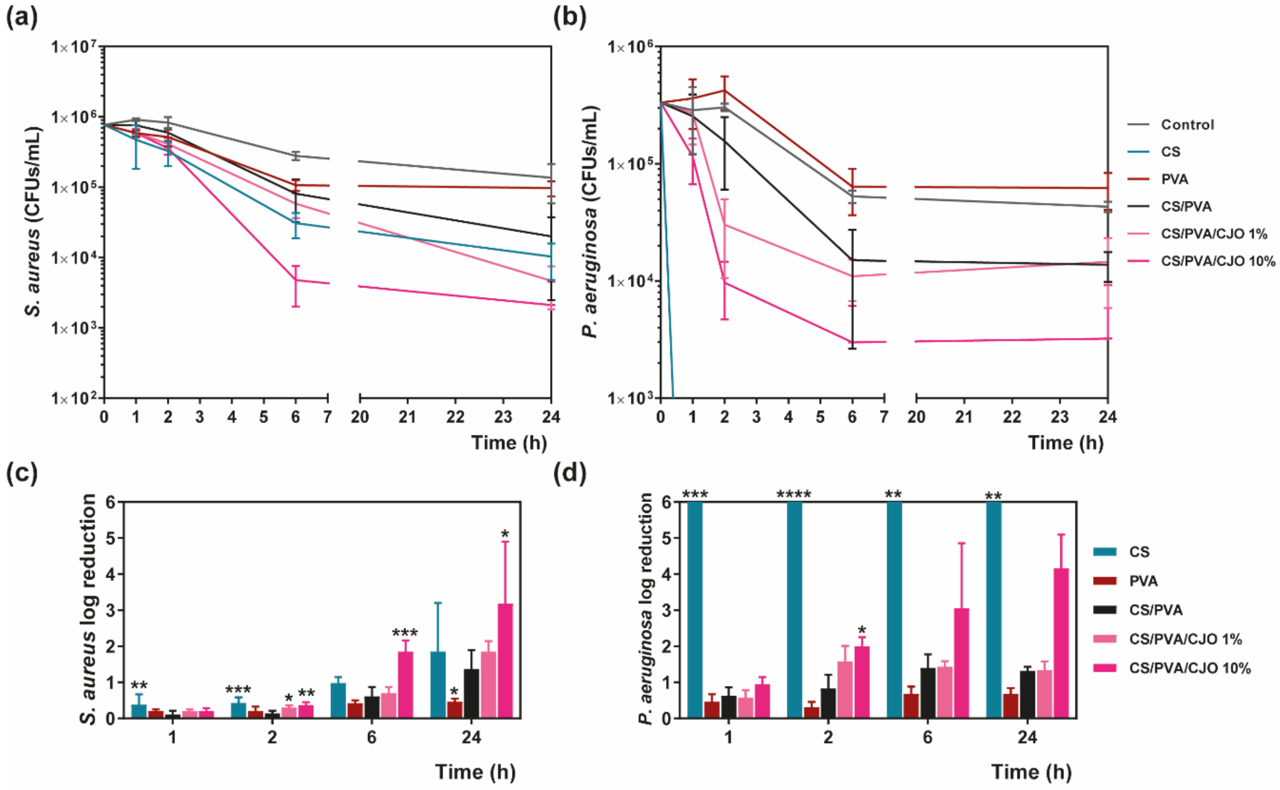
Antibacterial testing



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:

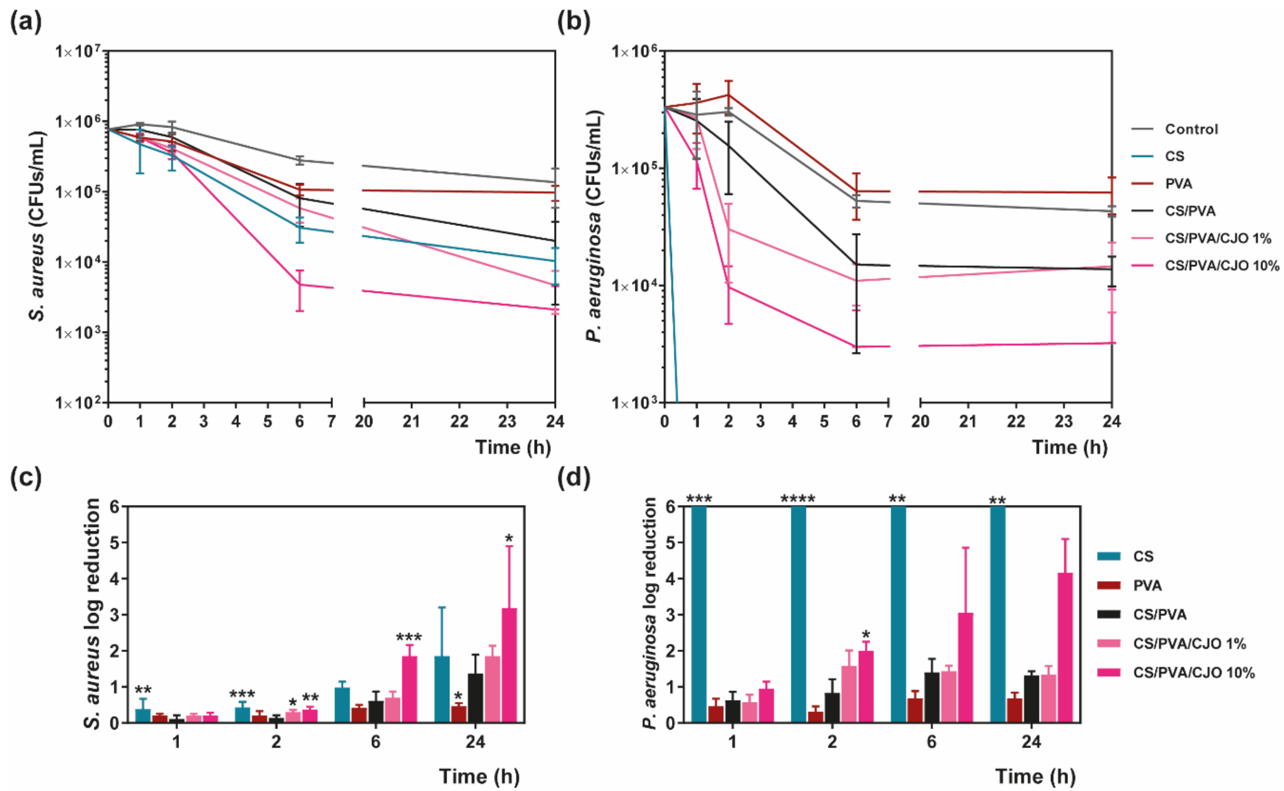
quickest AM action within 1h of incubation

P. aeruginosa:

complete bacterial elimination in 1h,

effect that endured until tested 24h

Antibacterial testing



CS/CLO/PVA film:

CS film:

the

Synergistic effect of CJO after adding it 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

- ✓ CS/PVA blended films were successfully built;
- ✓ Both CS and CJO show antibacterial activity against *S. aureus* and *P. aeruginosa;*
- ✓ CJO was successfully incorporated in the CS/PVA films at 1 and 10%wt;
- ✓ CJO-loaded CS/PVA films were 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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Co-P.I. Professor M. Teresa P. Amorim
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