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Antibacterial assessment of sodium alginate/gelatin films loaded with propolis extract

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Abstract: Problems associated with microbial resistance to antibiotics are growing due to their overuse. In this scenario, plant extracts have been considered as potential alternatives to antibiotics, since they can inhibit the action of the most common bacteria found colonizing infected wounds. The propolis extract (PE) has been used for centuries in folk medicine due to its antimicrobial, antioxidant, and anti-inflammatory properties as well as to its ability to induce tissue regeneration. Also known as "bee glue", propolis is a complex mixture of chemical constituents (such as resin, waxes, pollen, essential oil and organic compounds) with a high polyphenol content. To improve the stability and long-term effectiveness of PE in wound healing, polymeric films composed of biodegradable and biocompatible polymers are being engineered as delivery vehicles. Here, sodium alginate/gelatin (SA/GN) films (2 wt% SA concentration, polymer ratio 70/30 v/v), containing PE, were prepared via a simple, green process of solvent casting/phase inversion technique, followed by crosslinking with calcium chloride (2 wt%) solutions. The minimum inhibitory concentration (MIC) of PE was established as 0.338 mg/mL for *Staphylococcus aureus* and 1.353 mg/mL for *Pseudomonas aeruginosa*, the most prevalent bacteria in infected wounds. The extract was incorporated at *P. aeruginosa* MIC (a value effective against both bacteria) within the polymeric films before (blended with the polymeric solution) and after (immobilization via physisorption) film production. Flexible, highly hydrated films were obtained. Successful incorporation of PE was confirmed via Fourier-transformed infrared spectroscopy (FTIR). The antibacterial activity of the films was assessed via agar diffusion (qualitative) and killing time kinetics (quantitative) examinations. Data confirmed the modified films effectiveness to fight bacteria infections caused by *S. aureus* and *P. aeruginosa* and their ability to be applied in the treatment of infected wounds.

Keywords: antibacterial activity; plant extracts; propolis; localized drug release; bactericidal effects; infection control.

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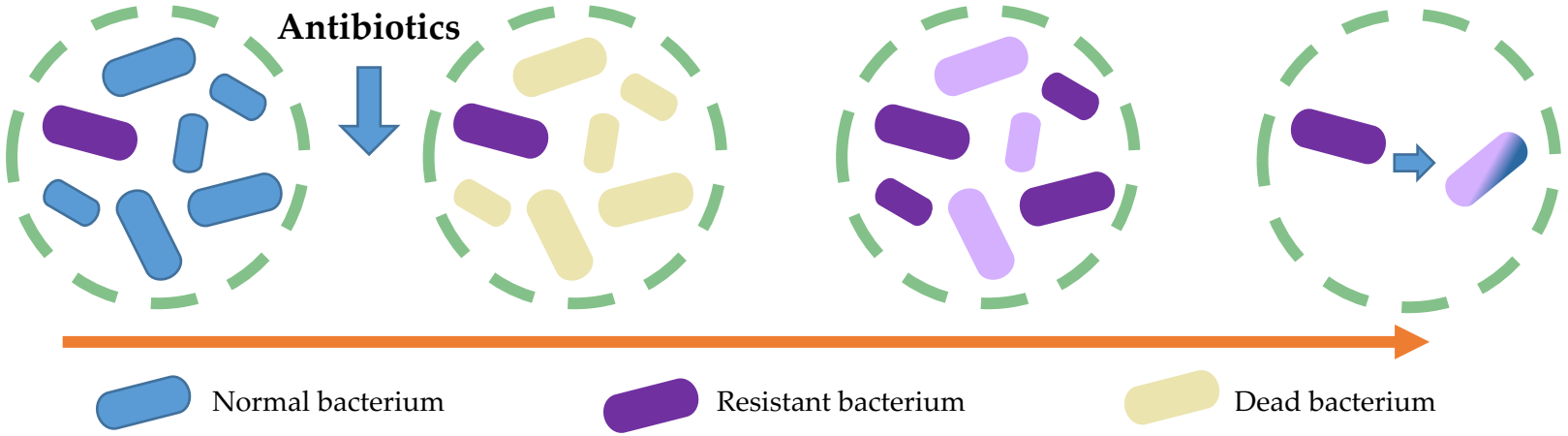
Antimicrobial resistance: a growing issue

How it happens?



High number of bacteria (a few of them are resistant to antibiotics)

Because of their genetic mutation, the resistant ones are not eliminated by antibiotics

Resistant bacteria multiply

Via DNA sequences exchange, the resistant bacteria can transmit their resistance to others

2.4 million people in Europe, North America and Australia will die from infections with resistant microorganisms in the next 30 years, causing a cost up to US$3.5 billion per year (OECD)

Solutions?

Biomolecules

Plant extracts

- Antimicrobial
- Antifungal
- Antiviral
- Regenerative properties

Propolis Extract (PE)

- Also known as ”bee glue”
- Complex mixture of chemical constituents
- Great antibacterial activity but non-toxic (humans and animals)
- Acts on the natural defenses, stimulating the imune system

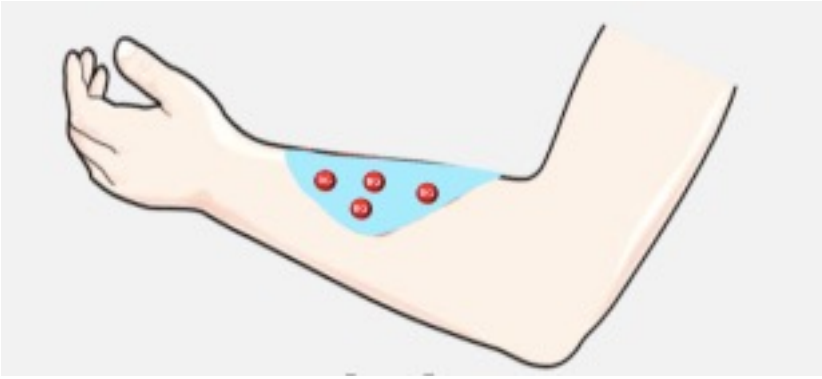
Solutions?

Hydrogel-like films

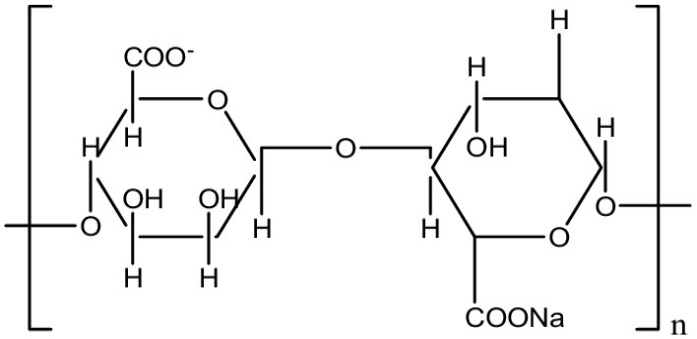
Wound dressings
2 in 1 solution

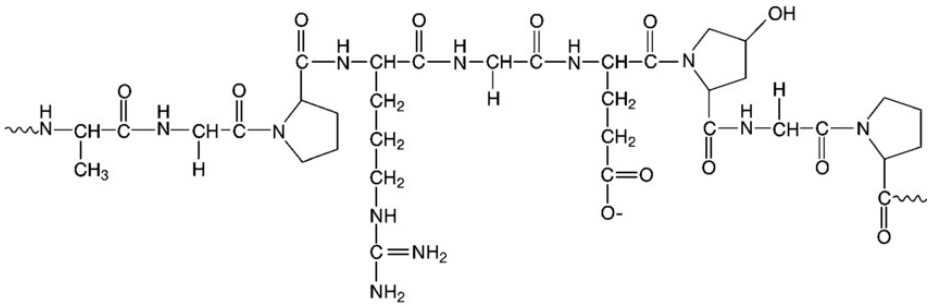
Protective barrier formed by biocompatible polymers

Antibacterial treatment due to PE presence



The polymer's choice is the key!





Sodium alginate (SA)

Gelatin (GN)

- Polysaccharide derived from brow seaweeds → Protein derived from collagen
- Water soluble, non-toxic, biodegradable and biocompatible → Water soluble, non-toxic, biodegradable and biocompatible
- Form hydrogel-like films when in contact with divalent cations Ca^{2+} → Contains many functional groups and cell binding sites in its structure

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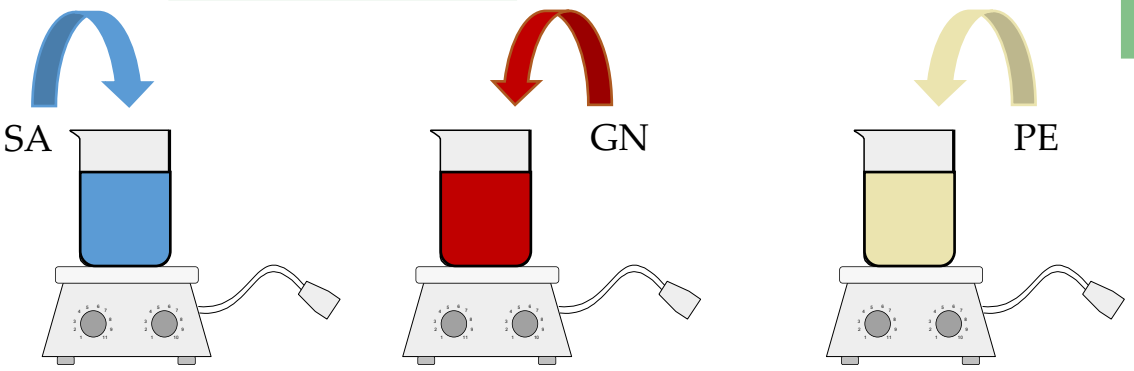
Solvent casting/phase Inversion

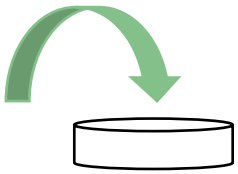
Polymers dissolution



Solvents: dH_2O
3h, 50 ºC, 200 rpm
Ratio: 70:30 SA/GN (SA 2wt%)

Solvent: dH_2O
Hydroalcoholic propolis extract (Drasanvi, Portugal)

Casting conditions



- Casting in Petri dishes
- 4 °C for 24 h
- Drying RT 6 days
- 1h at $CaCl_2$ (2 wt%)
- Wash at dH_2O 3 x 5 min

MICs of PE for *P. aeruginosa* and *S. aureus* bacteria were assessed via broth microdilution method

MICs (mg/mL)

	P. aeruginosa	*S. aureus*
PE	1.353	0.338

Incorporation in both conditions was performed at 1.6% v/v, a value expected to be efficient against both bacteria

Polymers dissolution → **Blending SA+GN** → **Casting Coagulation with $CaCl_2$** → **SA/GN**

Polymers and PE dissolution → **Blending SA+GN+ PE** → **Casting Coagulation with $CaCl_2$** → **SA/GN/PEb**

Polymers dissolution → **Blending SA+GN** → **Casting Coagulation with $CaCl_2$ Physisorption of PE** → **SA/GN/PEa**

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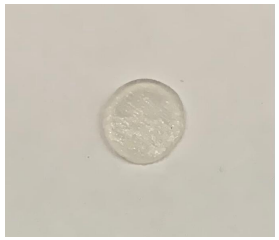
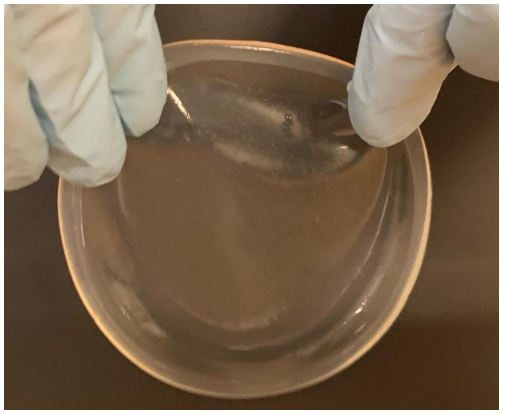
SA/GN



SA/GN/PEb

SA/GN/PEa



ATR-FTIR

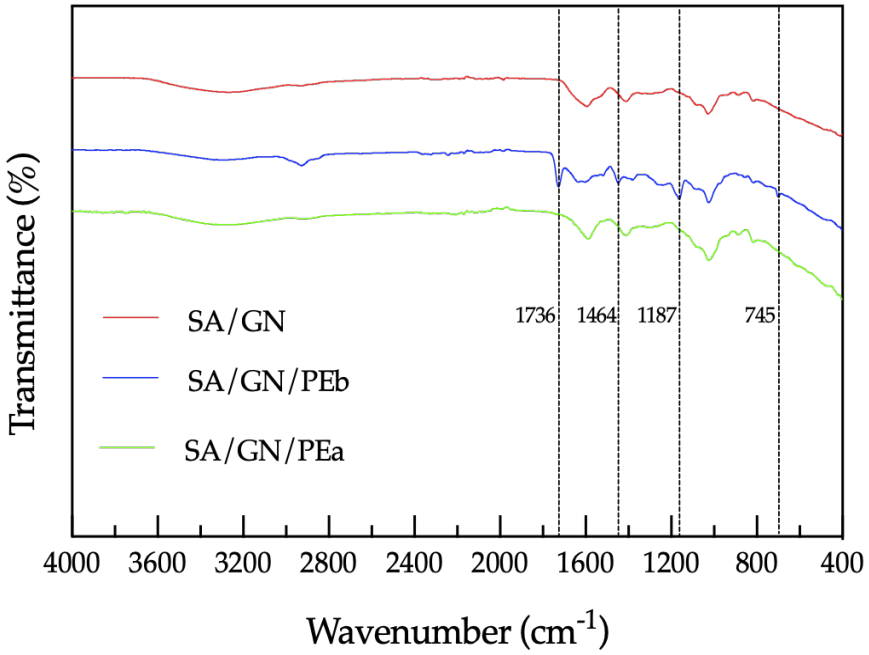


Figure 1. ATR-FTIR spectra of the SA/GN films unloaded and loaded with PE.

Water retention

Table 1. Water retention and average thickness of SA/GN loaded and unloaded films.

Sample	Water retention (%)	Thickness (mm)
SA/GN	2704.82	1.908
SA/GN/PEb	1776.33	1.095
SA/GN/PEa	2954.54	0.605

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Agar diffusion

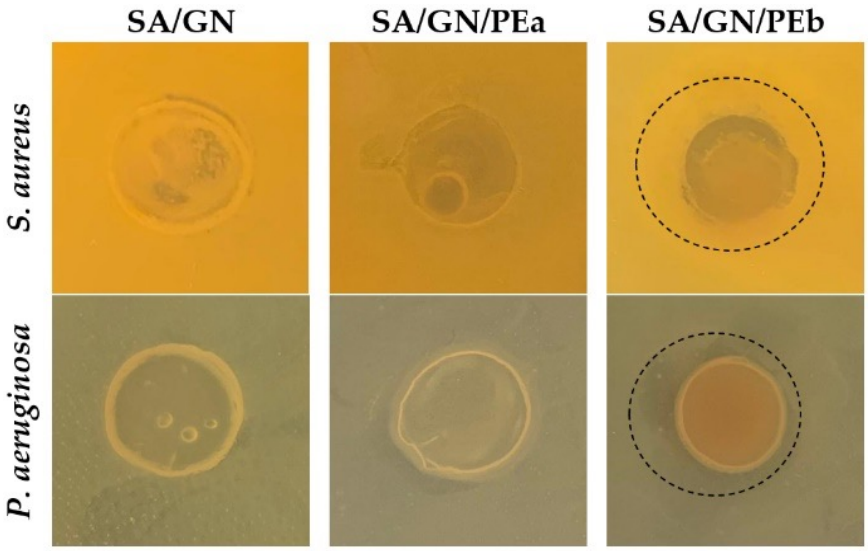

Figure 2. Propolis diffusion examinations from the loaded SA/GN films against S. aureus and P. aeruginosa bacteria cultured on solid media (agar).

Time-kill kinetics

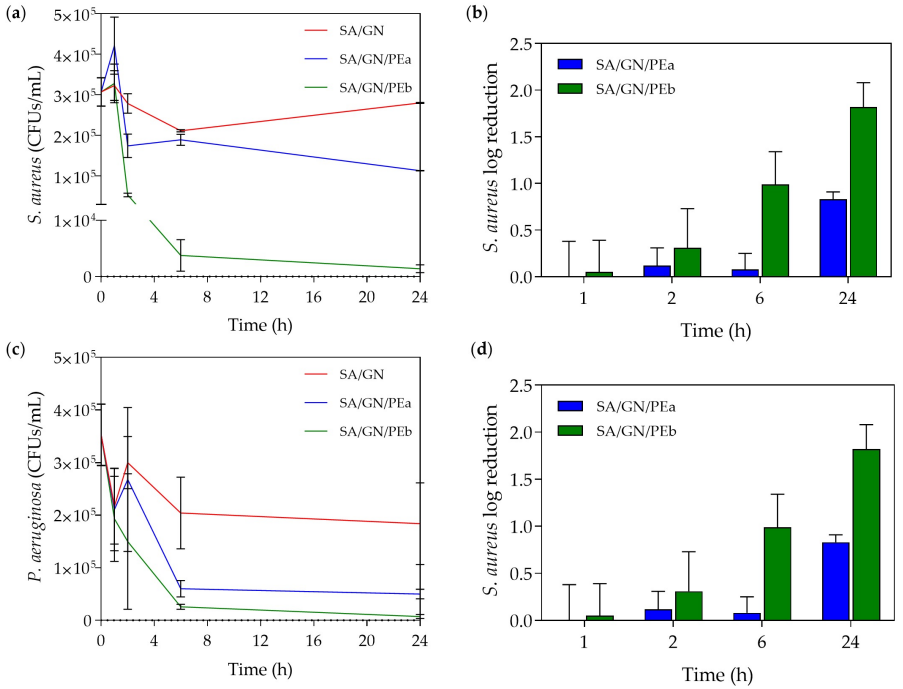

Figure 3. Time-kill kinetics of the SA/GN, SA/GN/PEa and SA/GN/PEb films, incubated from 1 h to 24 h, in contact with (**a**) *S. aureus* and (**c**) *P. aeruginosa* bacteria. Relative log reduction rates of the propolis loaded films compared to the control samples (SA/GN) against the (b) *S. aureus* and (d) *P. aeruginosa*.

The concentration of bacteria in the absence of PE, after 24h of growth, were 1.1×10^6 CFUs/mL for *S. aureus* and 1.8×10^6 CFUs/mL for *P. aeruginosa.*

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Conclusions

SA/GN films were successfully produced via solvent casting/phase inversion method.

PE incorporation within SA/GN/PEb films was confirmed by FTIR.

The SA/GN/PEa films showed higher water retention abilities and inferior antibacterial performance when compared to SA/GN/PEb films, thus leading to a conclusion that the incorporation of PE at blending solutions was more efficient.

The SA/GN/PEb films were able to inhibit *S. aureus* and *P. aeruginosa* growth in a more pronounced and sustained manner than SA/GN films, demonstrating that PE incorporation was beneficial for antibacterial purposes.

These results suggests that SA/GN/PE films could be used as alternatives to conventional treatments applied to infected wounds, for their improved bacterial inhibition.

Further studies aiming at evaluating the loading capacity and release behavior of PE from SA/GN/PE films in physiological media are ongoing.

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Thank you for your attention!

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