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Propolis loaded sodium alginate/gelatin films cross-linked with Ca^{2+} for potential wound dressing and healing applications

Chaired by **PROF. DR. VLADIMIR UVERSKY, DR. MARC MARESCA**
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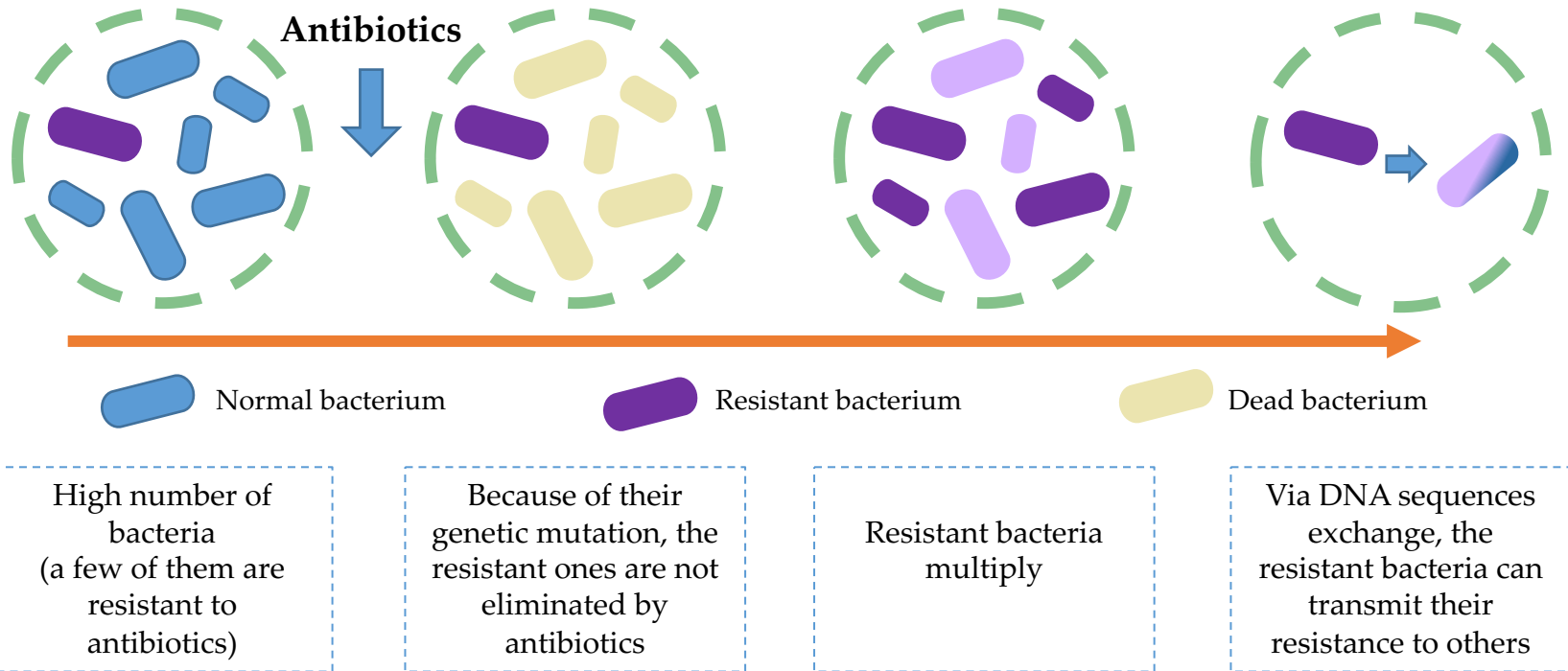
Abstract: Problems associated with microbial resistance to antibiotics are growing due to their overuse. In this scenario, plant extracts have been considered as potential alternatives to antibiotics, since they can inhibit the action of the most common bacteria found colonizing infected wounds. The 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.



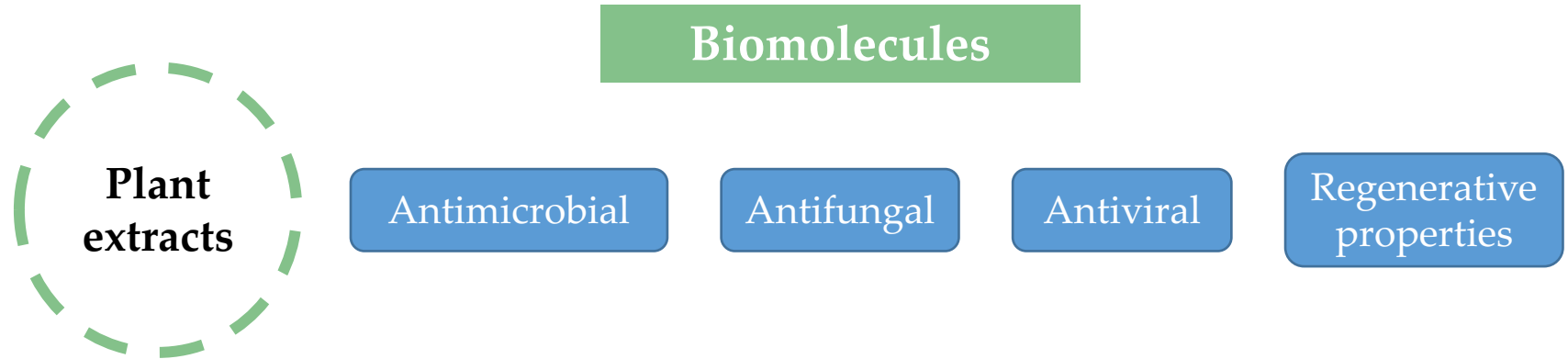
Antimicrobial resistance: a growing issue

How it happens?



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?



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 immune system

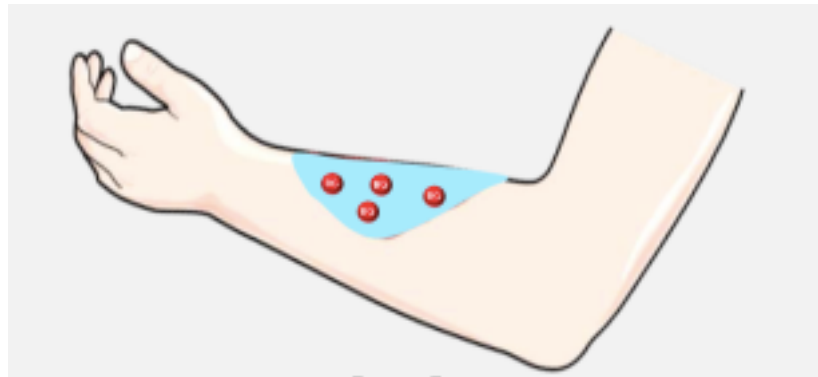
Solutions?

Hydrogel-like films

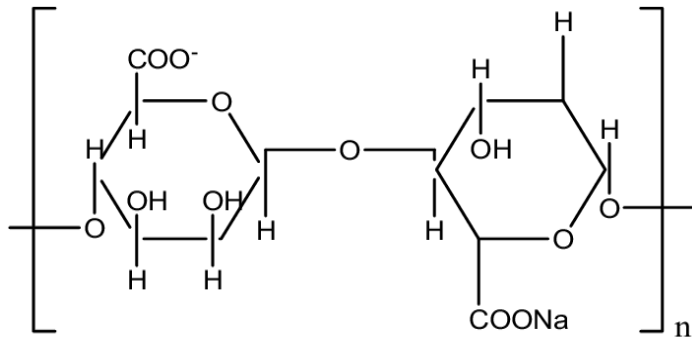
Wound dressings
2 in 1 solution

Protective barrier formed by
biocompatible polymers

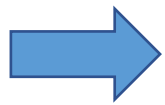
Antibacterial treatment due
to PE presence



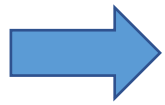
The polymer's choice is the key!



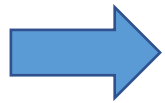
Sodium alginate (SA)



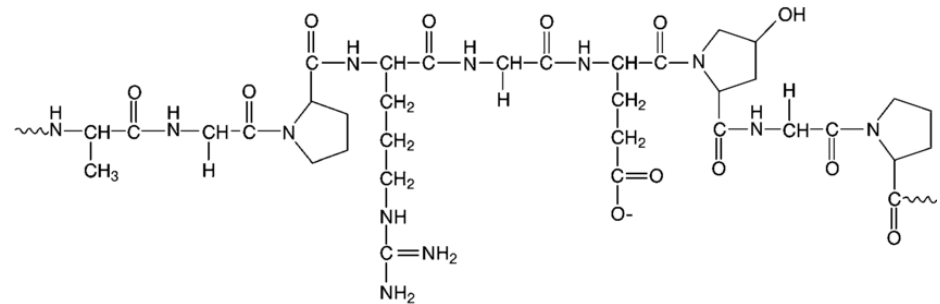
Polysaccharide derived from brown seaweeds



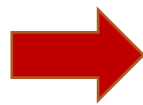
Water soluble, non-toxic, biodegradable and biocompatible



Form hydrogel-like films when in contact with divalent cations Ca²⁺



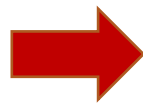
Gelatin (GN)



Protein derived from collagen



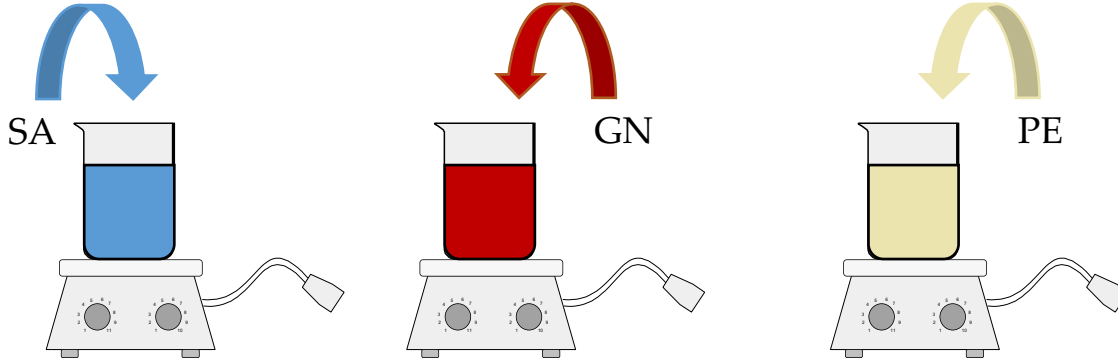
Water soluble, non-toxic, biodegradable and biocompatible



Contains many functional groups and cell binding sites in its structure

Solvent casting/phase Inversion

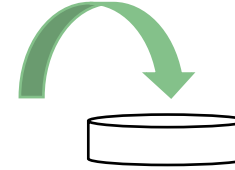
Polymers dissolution



Solvents: dH₂O
3h, 50 °C, 200 rpm
Ratio: 70:30 SA/GN (SA 2wt%)

Solvent: dH₂O
Hydroalcoholic propolis
extract (Drasanvi, Portugal)

Casting conditions



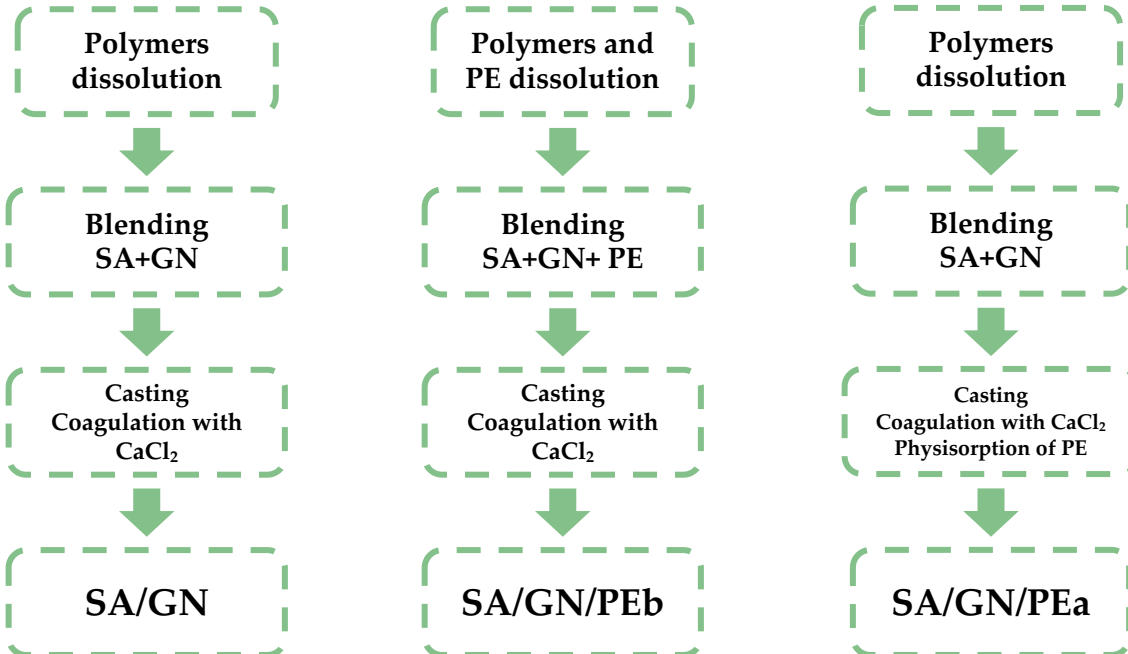
- Casting in Petri dishes
- 4 °C for 24 h
- Drying RT 6 days
- 1h at CaCl₂ (2 wt%)
- Wash at dH₂O 3 x 5 min

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

MICs (mg/mL)

	<i>P. aeruginosa</i>	<i>S. aureus</i>
PE	1.353	0.338

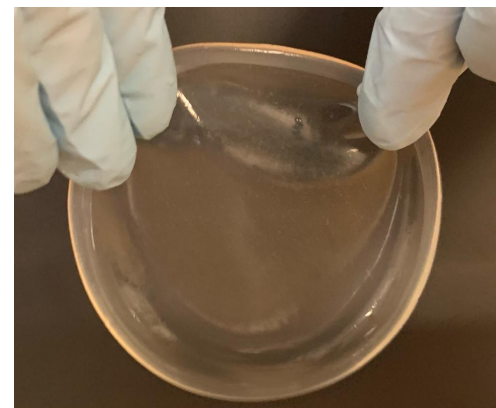
Incorporation in both conditions was performed at 1.6% v/v, a value expected to be efficient against both bacteria



SA/GN

SA/GN/PEb

SA/GN/PEa



ATR-FTIR

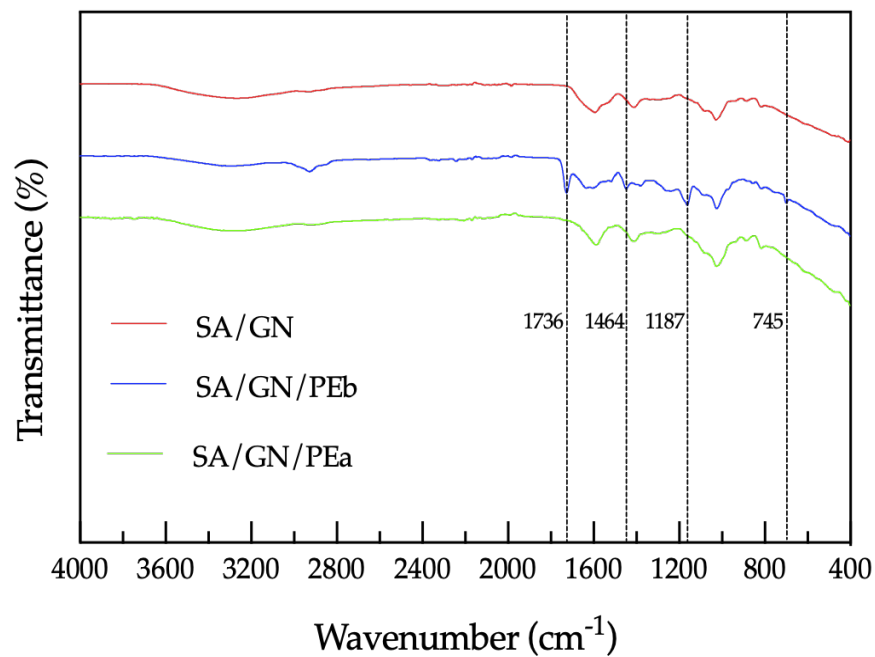


Figure 1. ATR-FTIR spectra of the SA/GN films unloaded and loaded with PE.

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

Agar diffusion

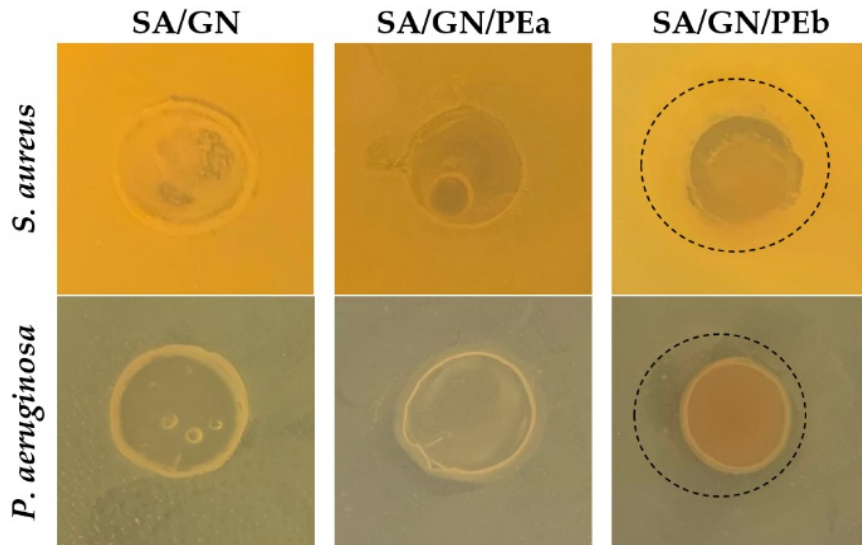


Figure 2. Propolis diffusion examinations from the loaded SA/GN films against *S. aureus* and *P. aeruginosa* bacteria cultured on solid media (agar).

Time-kill kinetics

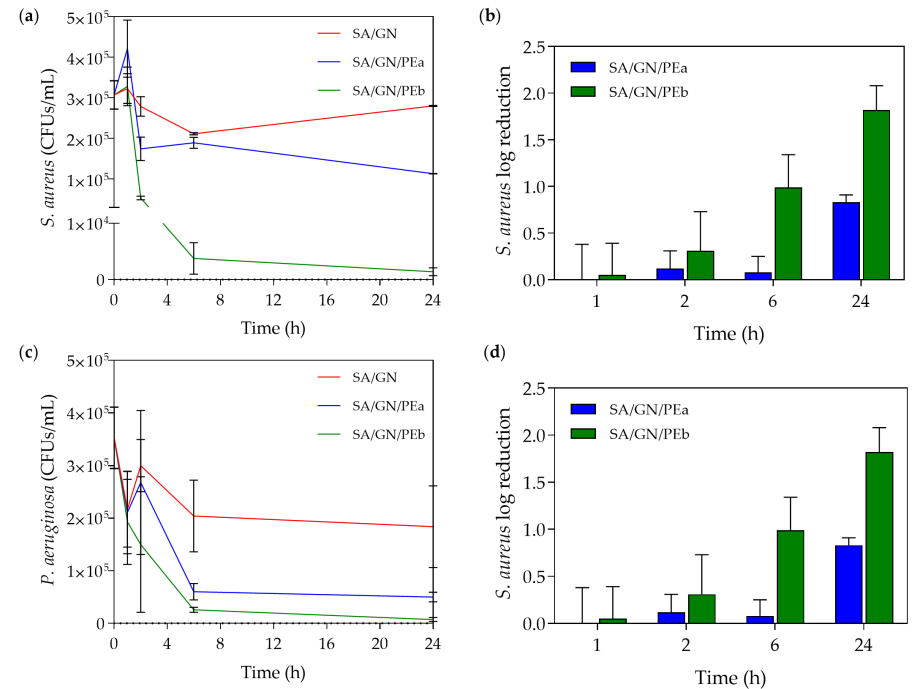


Figure 3. Time-kill kinetics of the SA/GN, SA/GN/PEa and SA/GN/PEb films, incubated from 1 h to 24 h, in contact with (a) *S. aureus* and (c) *P. aeruginosa* bacteria. Relative log reduction rates of the propolis loaded films compared to the control samples (SA/GN) against the (b) *S. aureus* and (d)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

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.

Acknowledgments

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