CENTRO DE CIÊNCIA E TECNOLOGIA TÊXTIL

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ANTIMICROBIAL ACTIVITY OF A BACTERIAL NANOCELLULOSE FILM FUNCTIONALIZED WITH NISIN Z FOR PROSPECTIVE BURN WOUNDS TREATMENT

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Introduction

Burn wounds can lead to numerous severe complications including bacterial infections that may cause patient morbidity and mortality, mostly in low- and middle-income countries [1]. The considerable increase in microbial resistance against traditional antibiotics is leading towards alternative strategies to treat bacterial infections [2]. Nisin Z is an antimicrobial peptide with a cationic character and an amphiphilic structure, which exhibits a significant antibacterial activity against Gram-positive bacteria [3]. Bacterial nanocellulose (BNC) is the most abundant polymer in nature and has been widely used as wound dressings [4]. Its impressive water retention capacity (> 99 %) and porosity are beneficial to manage wounds due to its potential to absorb exudates, providing a breathable and humid environment [5].

In this work, the functionalization of BNC with Nisin Z (BNC-NZ) via vacuum filtration is reported. The antimicrobial activity of BNC-NZ was evaluated against five of the most common bacteria found in burn wound infections.

Minimum Bactericidal Concentrations (MBCs) of Nisin Z

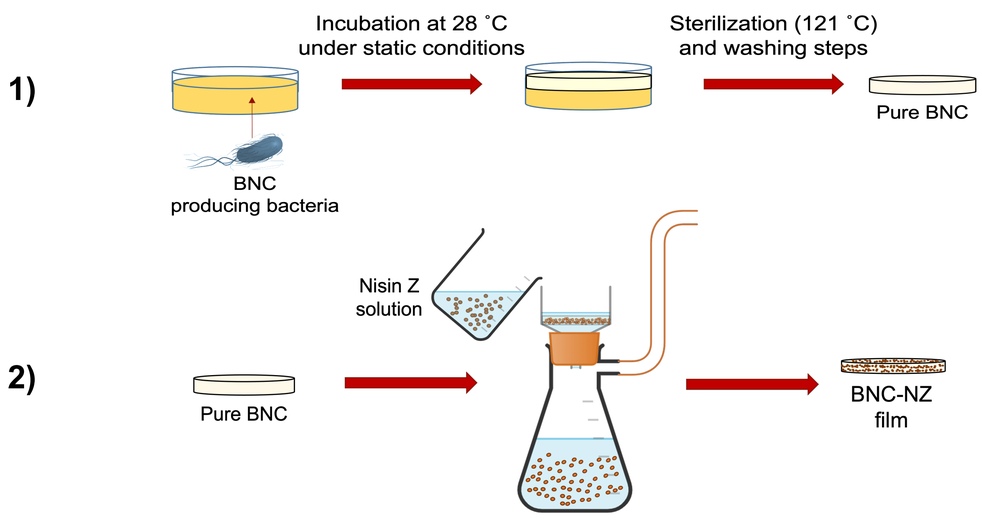
Initial Bacterial Concentration: 1x10^7 Colony Forming Units (CFUs)/mL in Mueller Hinton Broth (MHB)

Bacteria	MBC (µg/mL)
Staphylococcus aureus	16
Staphylococcus epidermidis	32
Escherichia coli	1024
Pseudomonas aeruginosa	*
Klebsiella pneumoniae	*

*No inhibition was verified at the maximum Nisin Z concentration used (1024 µg/mL).

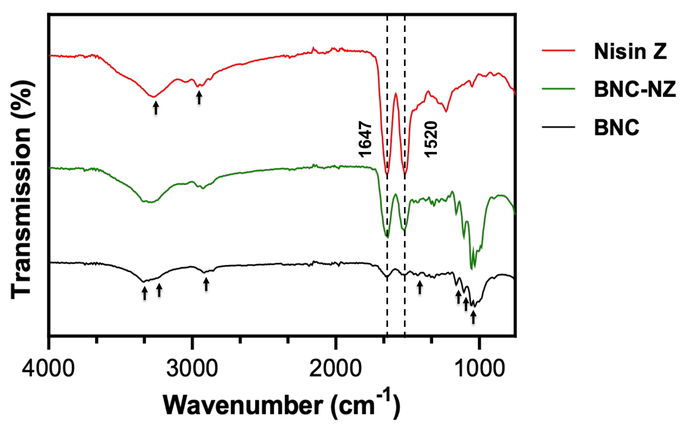
Processing and Functionalization of BNC Film with Nisin Z

Initial Nisin Z Loading: 10 times highest MBC



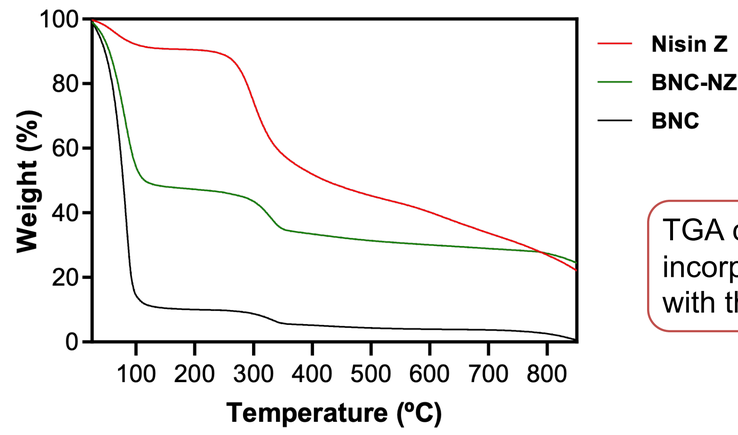
Morphological and Thermal Characterization of BNC-NZ

1) Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Analysis



Nisin Z		BNC	
Wavenumber (cm^{-1})	**Peak attribution**	**Wavenumber (cm^{-1})**	**Peak attribution**
3300	-OH asymmetrical stretching	3350	-OH stretching vibration
3000	-CH symmetrical stretching	2890	-CH stretching of aliphatic CH_2
1647	Amide group	1650	Bending mode of adsorbed H_2O
1520	Bending primary amines	1550	Carboxylic groups
		1060	C-O-C stretching

2) Thermal Gravimetric Analysis (TGA)



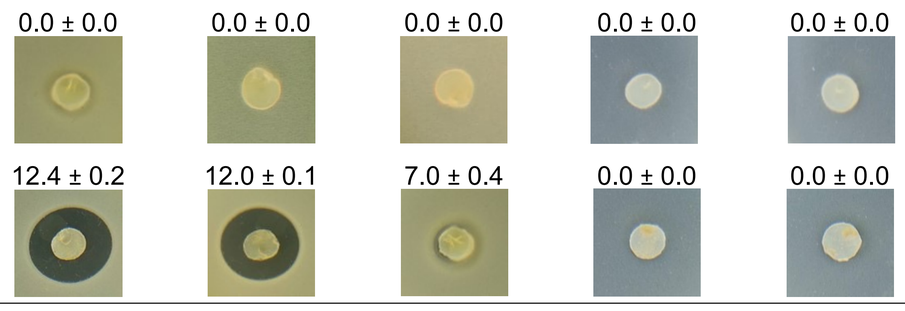
TGA curves suggest that Nisin Z incorporation did not interfere with the degradation of BNC.

Antimicrobial Activity of BNC-NZ

1) Agar Diffusion Assay

Initial Bacterial Concentration: 1x10^7 CFUs/mL in Tryptic Soy Broth (TSB) or Nutrient Broth (NB)

Zone of Inhibition diameter (mm)	Bacteria				
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
BNC	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BNC-NZ	12.4 ± 0.2	12.0 ± 0.1	7.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.0

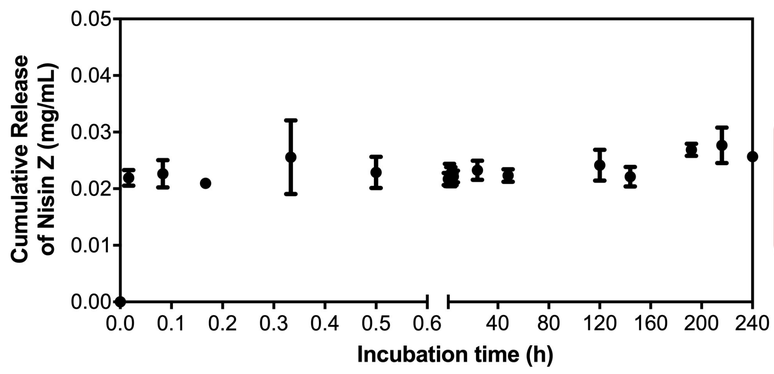


2) Shake Flask Assay

Initial Bacterial Concentration: 1x10^7 CFUs/mL in TSB or NB

Bacteria	Bacterial inhibition (%)
S. aureus	≈ 99
S. epidermidis	≈ 97
E. coli	≈ 20
P. aeruginosa	0
K. pneumoniae	0

Nisin Z Release Kinetics



Total released amount of 6 % after burst release at the initial 3 minutes, followed by a sustainable release.

Conclusions and Future Perspectives

- Vacuum filtration method proved efficient in BNC functionalization with Nisin Z;
- Against Gram-positive bacteria, BNC-NZ showed a good antibacterial activity;
- To improve the antibacterial activity against Gram-negative bacteria, different approaches, namely surfactants, will be used;
- Physical properties such as permeability and stability in wound exudates, and mechanical testing such as draping and pure bending, will be evaluated;
- Overall, the data revealed the potential of BNC-NZ for prospective application in burn wound dressings.

References

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Acknowledgments

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COMPETE

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