UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

IARIA

Polysaccharide-based materials with antibacterial properties

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- Master degree at UCT Prague – Drug production, 2019
- Summer 2019 – internship at Manipal Institute of Technology (MIT) in India
- Ph.D. at UCT Prague – Drugs and Biomaterials
- Publications
  - KUTOVÁ A., STAŇKOVÁ L., VEJVODOVÁ K., KVÍTEK O., VOKATÁ B., FAJSTAVR D., KOLSKÁ Z., BROŽ A., BAČÁKOVÁ L., ŠVORČÍK V. Influence of Drying Method and Argon Plasma Modification of Bacterial Nanocellulose on Keratinocyte Adhesion and Growth. *Nanomaterials*, **2021**, vol. 11.
  - KUTOVÁ A., KVÍTEK O., HURTUKOVÁ K., ŠVORČÍK V. Preparation of Bacterial Nanocellulose-Hydroxyapatite Composite. *Advanced Materials Letters*, **2022**, vol. 13.
  - PETRUSONYTE G., KUTOVÁ A., GRAUZELIENE S., OSTRAUSKAITE J. Optimization of vanillin bis epoxy coating properties by changing resin composition and photocuring conditions. *Polymer Bulletin*, **2022**.

Bacterial nanocellulose (BNC)[1]

Chitosan (Chit) [2]

Produced by some particular bacterial strains

Its fibre diameter does not exceed 100 nm

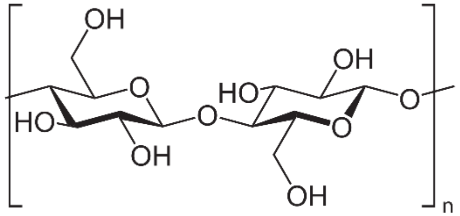
Hydrophilic, biocompatible

Excellent mechanical properties, high porosity, high crystalinity

Deacetylation of chitin from cruscaceans shells

Non-toxic, biocompatible, promotes wound healing

Antibacterial properties



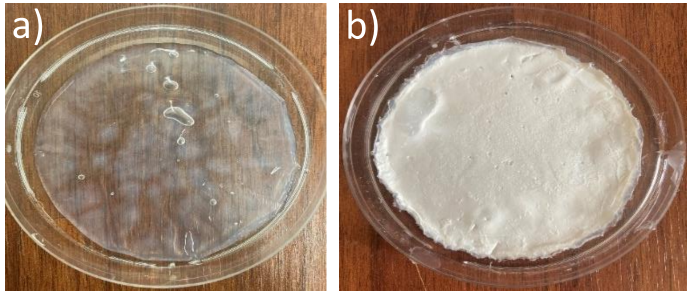
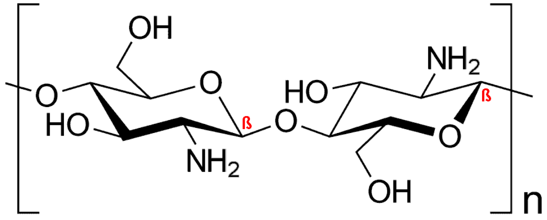


Fig 1: a) Harvested and washed BNC b) lyopohilized BNC

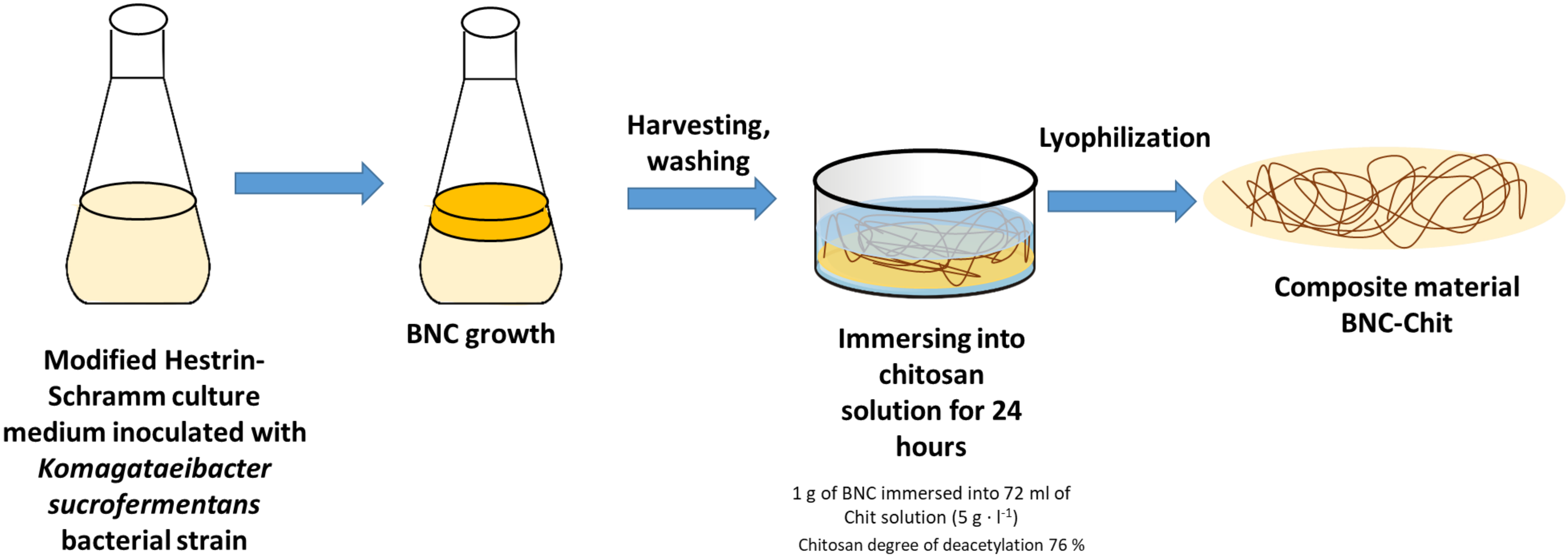


Composite material: Suitable for **antibacterial wound dressing** or **food packaging**

!!! The chitosan content could negatively affect some properties (e.g. mechanical[3] or biological[4]) !!!

[1] Klemm, D.; et al. Nanocelluloses: A New Family of Nature-Based Materials. Angew. Chem. Int. Ed. **2011**, *50*, 5438.
[2] Matica, M. A.; et al. Chitosan as a Wound Dressing Starting Material: Antimicrobial Properties and Mode of Action. Int. J. Mol. Sci. **2019**, *20*, 5889.
[3] Yin, N.; et al. Characterization of antibacterial bacterial cellulose composite membranes modified with chitosan or chitooligosaccharide. *Carbohydr. Polym.* **2020**, *229*, 115520.
[4] Lin, W.-C.; et al. Bacterial cellulose and bacterial cellulose–chitosan membranes for wound dressing applications. Carbohydr. Polym. **2013**, *94*, 603.

Graphical abstract



Composite preparation

Chitosan content: approx 17% (w/w)

FTIR[1,2]

- 3346 – OH stretching
- 3290 – NH stretching
- 2900 – CH stretching
- 1649 – amide I
- 1537 – amide II
- 1300-1500 – C-C-H, C-O-H bending vibrations
- 1100-1300 – glycosidic bond
- 950-1100 – C-OH
- 600-900 – glucose cycle

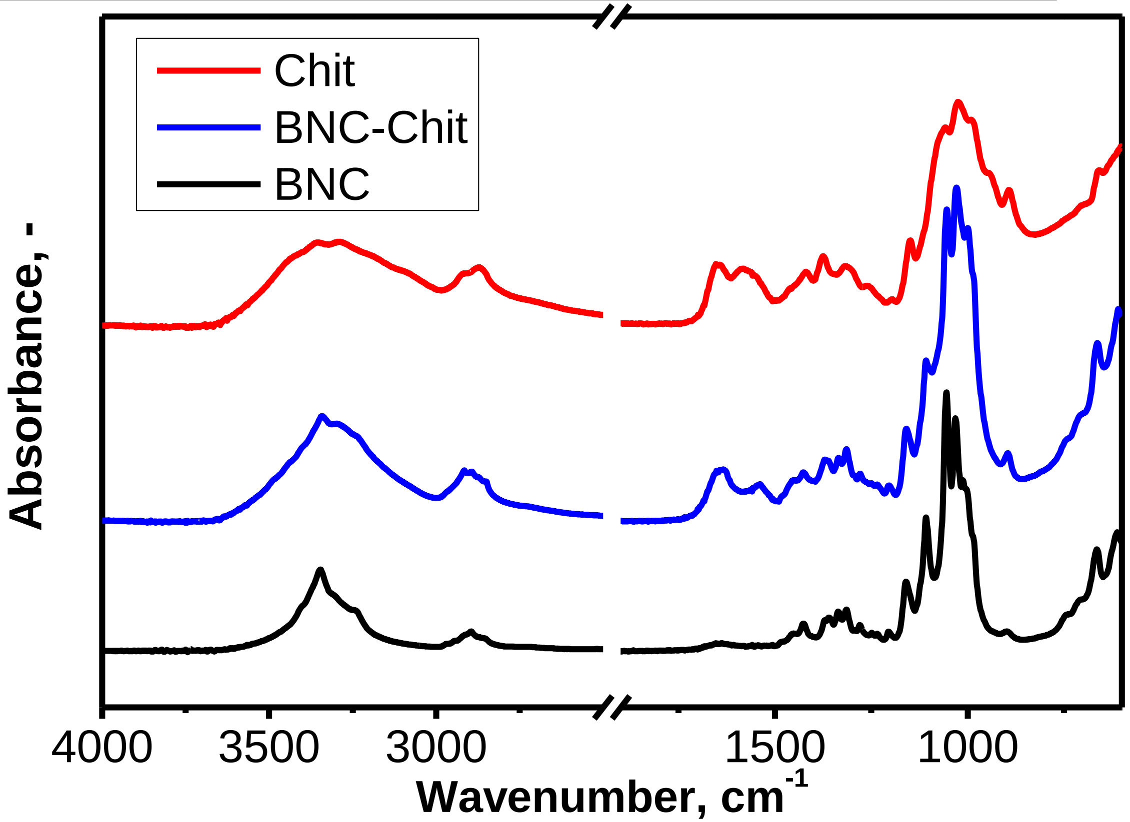


Fig 2: FTIR spectrum of Chit (red), prepared composite BNC-Chit (blue) and BNC (black)

[1]Kutová, A.; et al. Influence of Drying Method and Argon Plasma Modification of Bacterial Nanocellulose on Keratinocyte Adhesion and Growth. *Nanomater.* **2021**, *11*, 1916.

[2]Queiroz, M. F.; et al. Does the Use of Chitosan Contribute to Oxalate Kidney Stone Formation? *Mar. Drugs* **2015,** *13,* 141.

Surface morphology

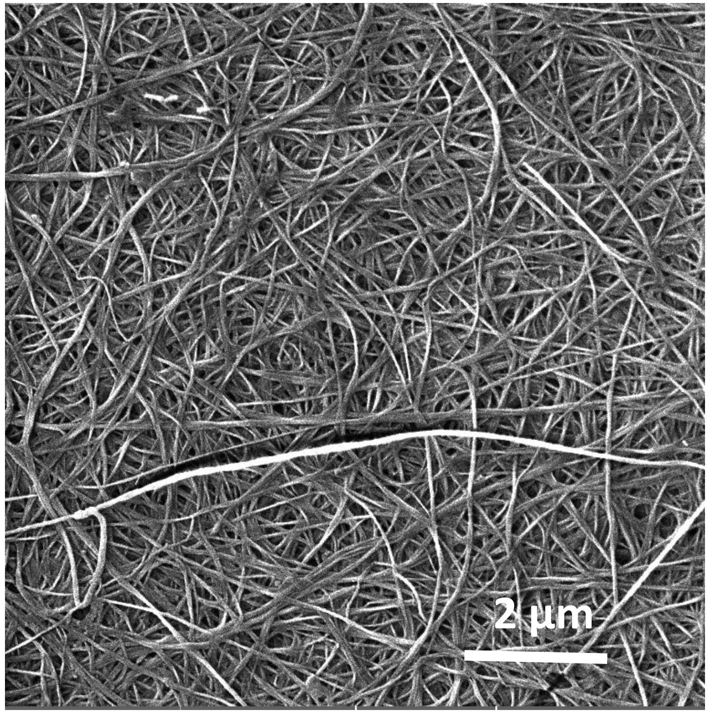


Fig 3: SEM image of pure BNC showing the nanostructure of the material with present pores.

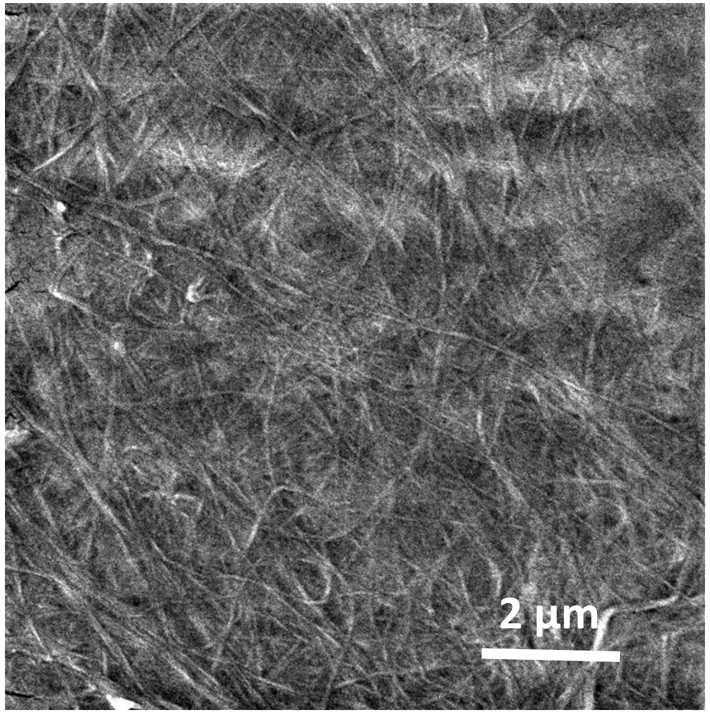
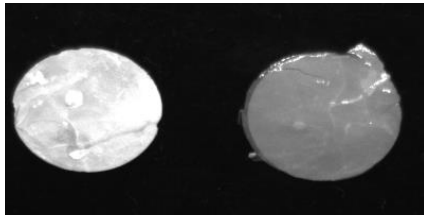


Fig 4: SEM image of BNC-Chit composites showing the preserved nanostructure of BNC. However its pores are clogged with Chit.

Fig 4 shows the penetration of chitosan into the nanostructure of cellulose fibres in the BNC-Chit composite leading to a less porous surface and less pronounced fibrous character compared to pure BNC at Fig 3.

Mechanical and swelling properties

	BNC	BNC-Chit	BNC lit.[1]	BNC-Chit lit.[1]
Chitosan solution ($c \cong 0.5\ g \cdot L^{-1}$)	-	5 mL	-	100 mL
Loading velocity	$10\ mm \cdot min^{-1}$		$1\ mm \cdot min^{-1}$	
Young´s modul [MPa]	39 ± 7	29 ± 12	83 ± 7	341 ± 37
Elongation [%]	9.1 ± 1.2	7.7 ± 2.4	4.7 ± 0.7	1.3 ± 0.3
Tensile strenght [MPa]	3.6 ± 0.8	2.1 ± 0.9	4.5 ± 1.0	3.8 ± 0.5
Swelling ratio [%]	7790 ± 730	2600 ± 690	8414	1714



[1] Yin, N.; et al. Characterization of antibacterial bacterial cellulose composite membranes modified with chitosan or chitooligosaccharide. *Carbohydr. Polym.* **2020**, *229*, 115520.

Antibacterial properties against *Staphylococcus aureus* (SA)

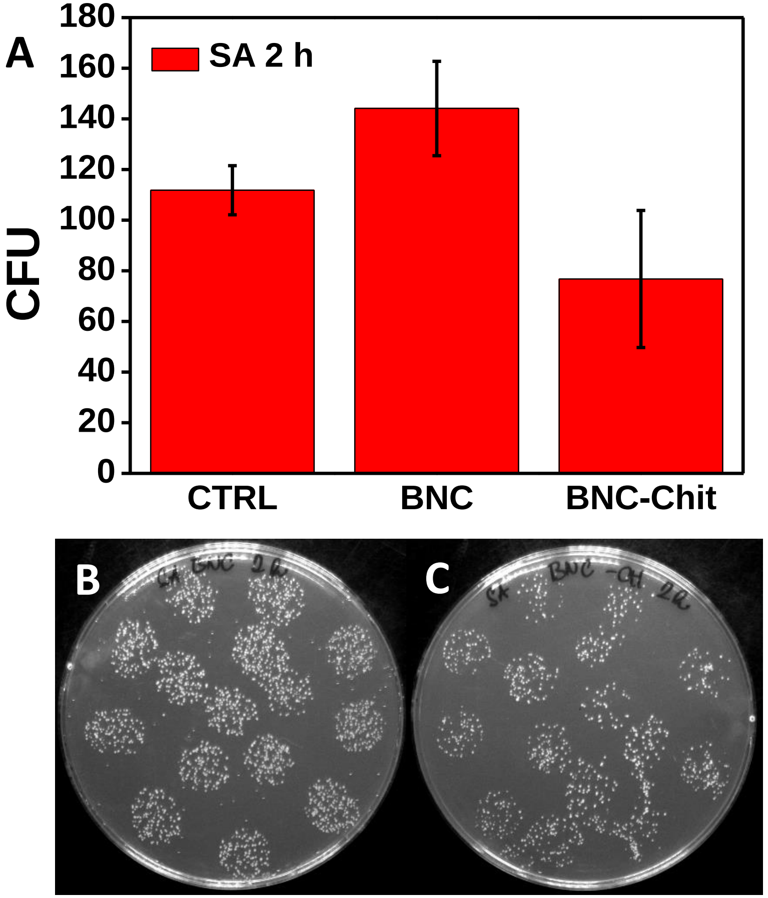


Fig 5: A) BNC and BNC-Chit antibacterial aktivity after 2 hours agains *S. aureus* B) drop-plate method for BNC C) drop-plate method for BNC-Chit

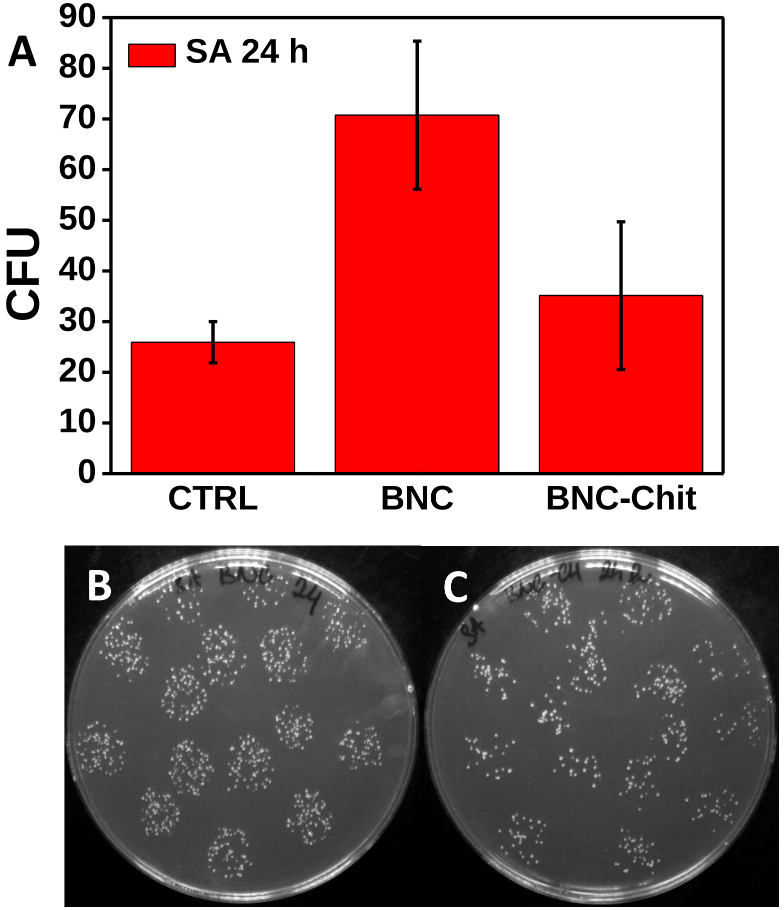


Fig 6: A) BNC and BNC-Chit antibacterial aktivity after 24 hours agains *S. aureus* B) drop-plate method for BNC C) drop-plate method for BNC-Chit

These graphs show the antibacterial activity of BNC-Chit composite towards the SA bacterial strain. However, the bacteria profit from the BNC itself leading to an increase of CFUs compared to CTRL.

CFU: Colony forming unit
CTRL: Control
BNC: Bacterial nanocellulose
BNC-Chit: BNC-chitosan composite

Antibacterial properties against *Escherichia Coli* (EC)

CFU: Colony forming unit
CTRL: Control
BNC: Bacterial nanocellulose
BNC-Chit: BNC-chitosan composite

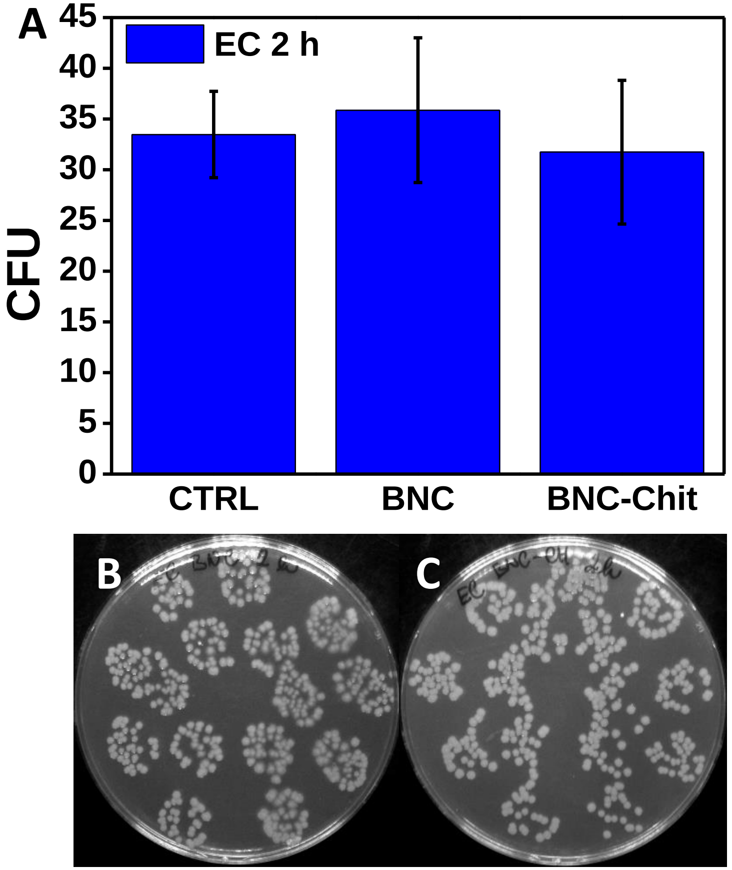
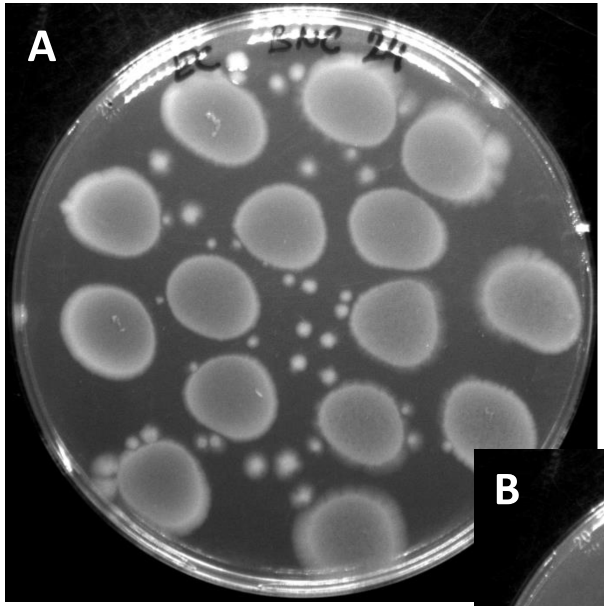


Fig 7: A) BNC and BNC-Chit antibacterial aktivity after 2 hours agains *E. coli* B) drop-plate method for BNC C) drop-plate method for BNC-Chit



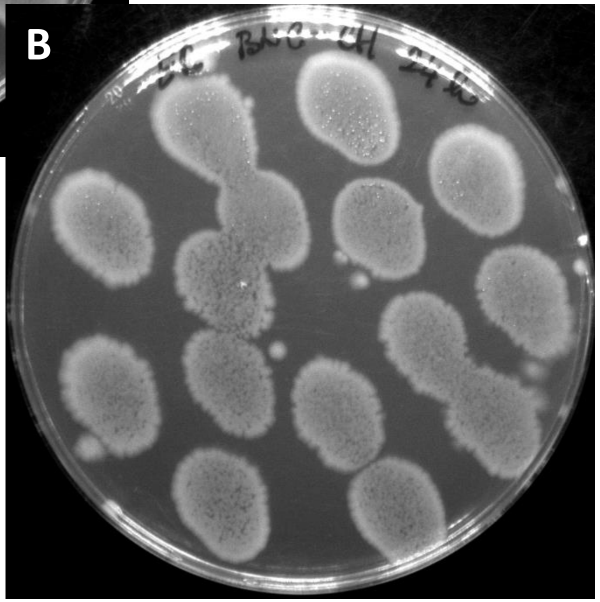


Fig 8: A) drop-plate method for BNC B) drop-plate method for BNC-Chit
The number of CFUs after 24 hours was not countable. However, the decrease in the number of CFU for the composite is visible.

Main results

The composite material BNC-Chit was prepared with preserved nanostructure with Chit in its pores.

The addition of Chit led to a slight decrease in mechanical properties but significant embrittlement did not occur.

The clogged pores of chitosan caused a significant decrease in the swelling ratio.

The BNC-Chit showed high antibacterial activity towards gram-positive bacteria.

Future: → Higher concentration of chitosan to provide better antibacterial properties against gram-negative bacteria but with preserved mechanical properties.

→ Cell adhesion studies.

➡ Material suitable for biomedical applications or as packaging in the food industry.

Thank you for your attention