



# Alginate based microcapsules for local probiotic delivery: Evaluation of bacterial entrapment, release, and growth

Janja Mirtič

T. Rijavec, Š. Zupančič, A. Lapanje, J. Kristl  
Faculty of Pharmacy, University of Ljubljana, Slovenia  
Institute Josef Stefan, Ljubljana, Slovenia

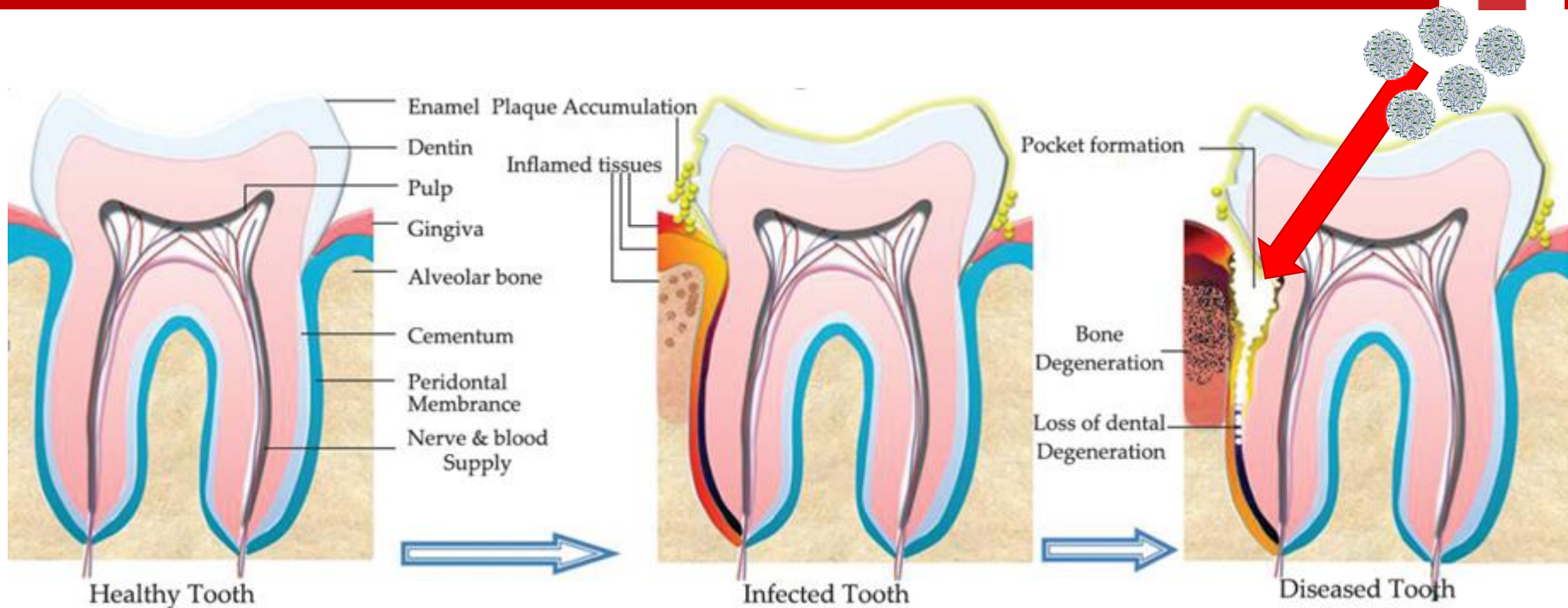
# Outline



- Introduction
  - Probiotics for periodontal disease treatment
  - Microcapsules as probiotic delivery system
- Methods of production and characterization
- Results
  - Physico-chemical characterization
  - Biological characterization
- Key message



# Periodontal disease



BACTERIAL INFECTION of a PERIODONTAL POCKET



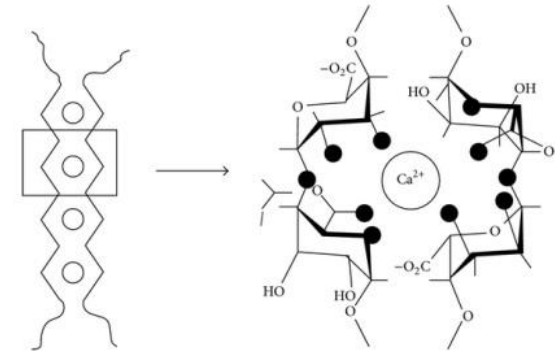
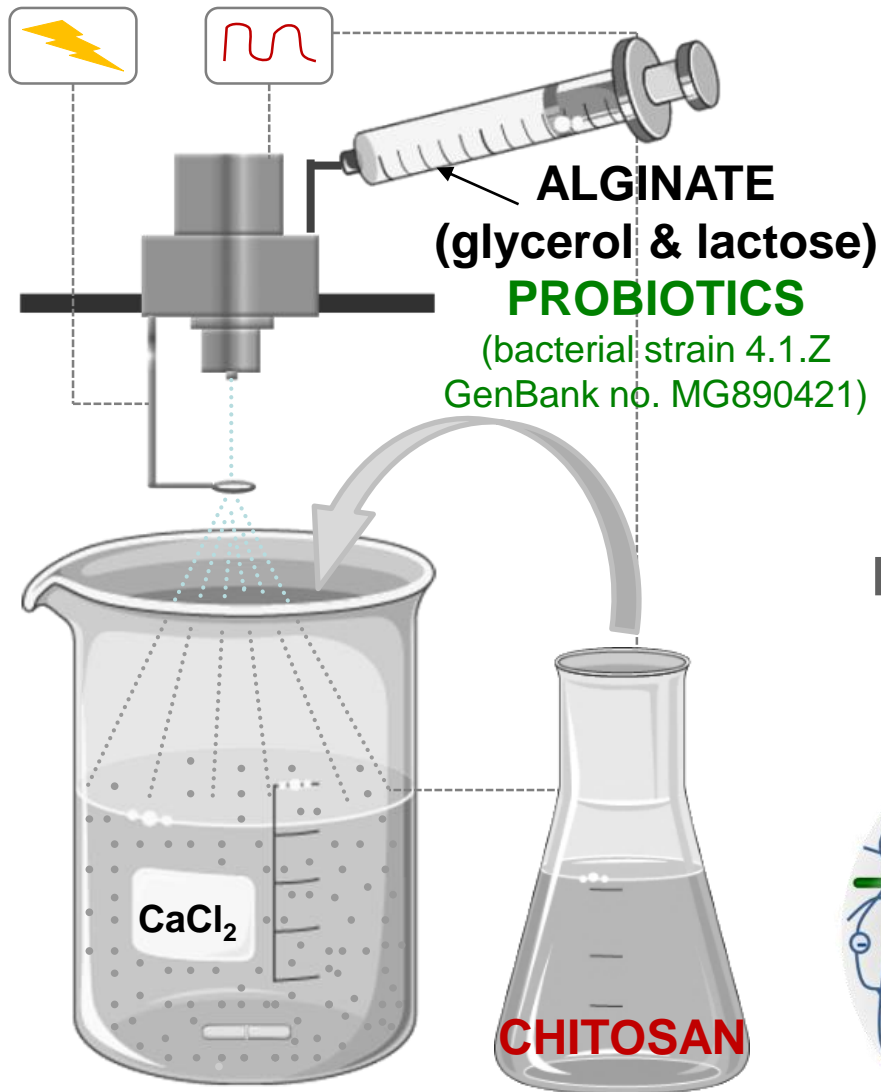
Microbial imbalance and localized inflammatory response



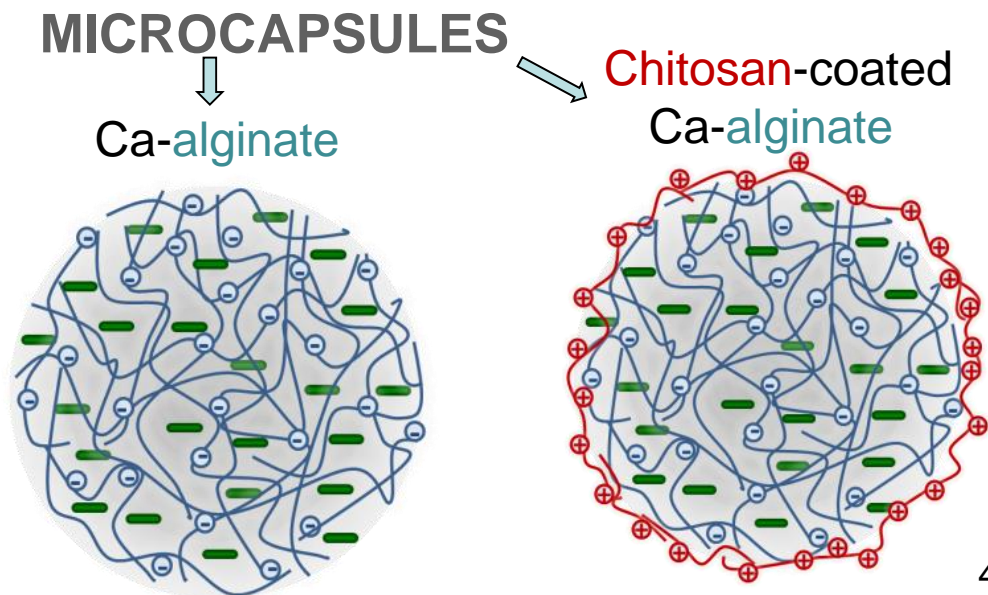
**Local therapy with probiotics**



# Microcapsules as probiotic delivery system



Alginate - Ca<sup>2+</sup> complexation:  
The „egg-box“ model



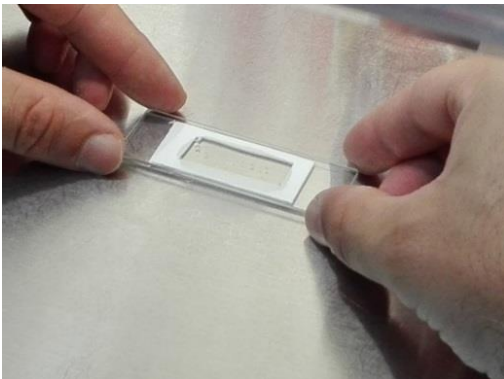


# Methods of characterization

- Size and morphology of microcapsules
  - Optical microscopy
  - Scanning electron microscopy
  - Laser diffraction method (hydrodynamic behaviour)
- Viscosimetric assay of bioadhesion
- Biological characterization
  - Enumeration of bacteria and viability during storage
  - Spatial cell distribution and time-lapse microscopy (agar chambers)
  - Growth kinetics
  - Antimicrobial activity testing



# Microscope agar chambers



- Microscope slide + rectangular double-sided sticky frame + glass cover slip
- Hot Brain Heart Infusion agar medium pipetted into the frame flattened by a second microscope slide and left to solidify at 4°C
- Top microscope slide removed, the dispersed microcapsules pipetted onto the surface of the agar and the top cover slip fixed on top of the sticky frame to seal the agar chamber
- Observation under fluorescent or optical microscope



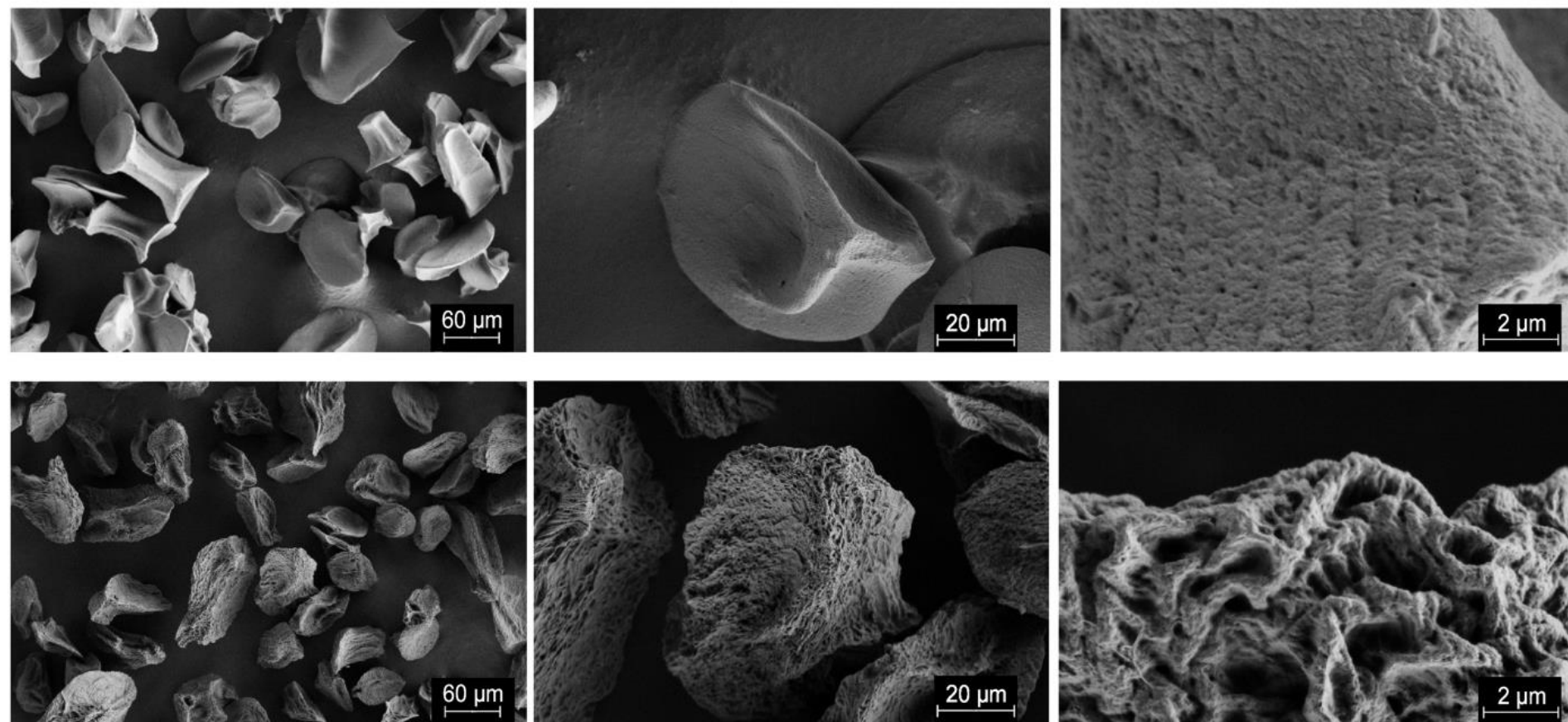
# RESULTS





# Microcapsule size and morphology

Magnification



**Figure 1** SEM images at increasing magnification showing differences in surface morphology for Ca-alginate microcapsules (upper panel) and chitosan-coated Ca-alginate microcapsules (lower panel).

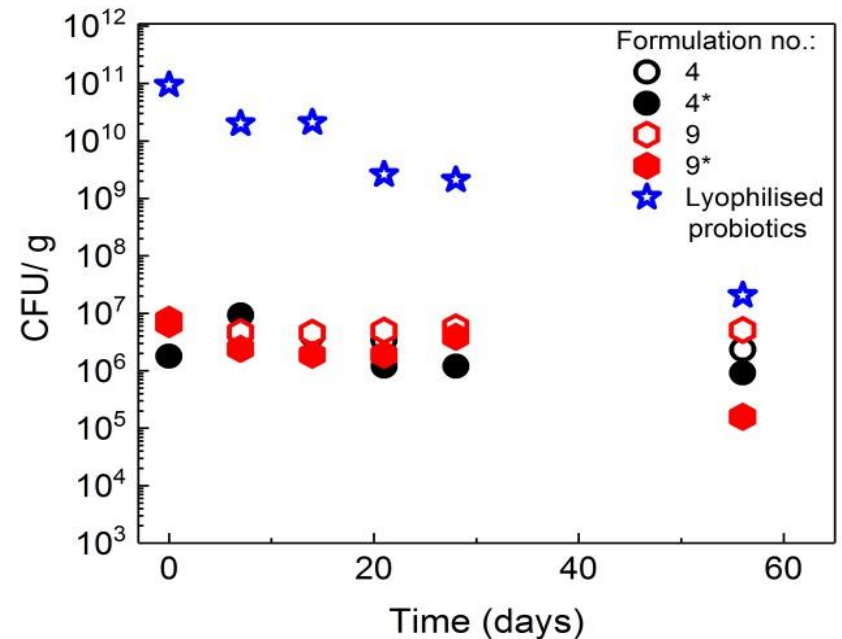


# Quantification of encapsulated bacteria

## ENCAPSULATION

- **Vegetative cells:**  
Up to  $10^8$  of CFU/g of dried microcapsules
- **Spores:**  
Up to  $10^{10}$  of CFU/g of dried microcapsules

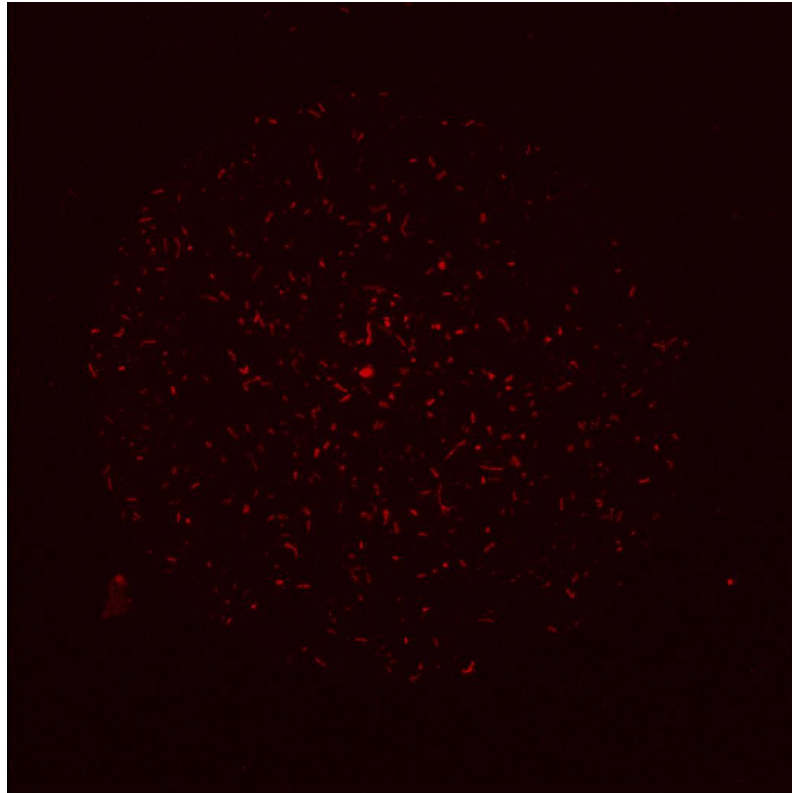
## STABILITY DURING STORAGE



**Figure 2** Storage stability of encapsulate vegetative cells compared to lyophilized probiotics alone (☆) during 2 months at 4°C.



# Spatial distribution of cells in microcapsule

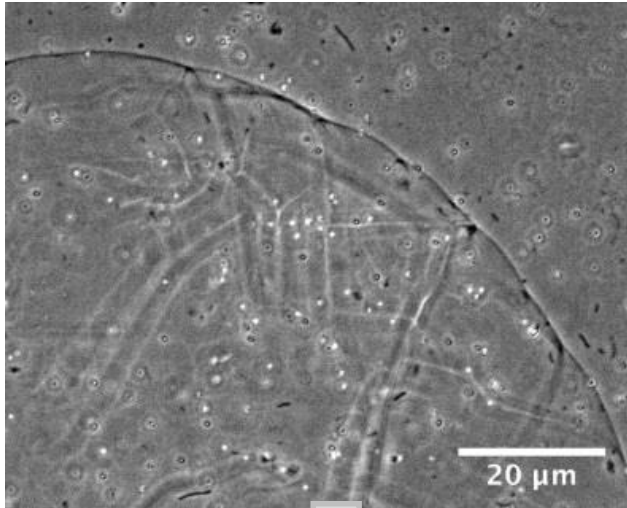


**Video 1** Distribution of cells in hydrated chitosan-coated alginate microcapsule. Video represents the 3D reconstruction of the z-stack images (36 frames, 5 fps).

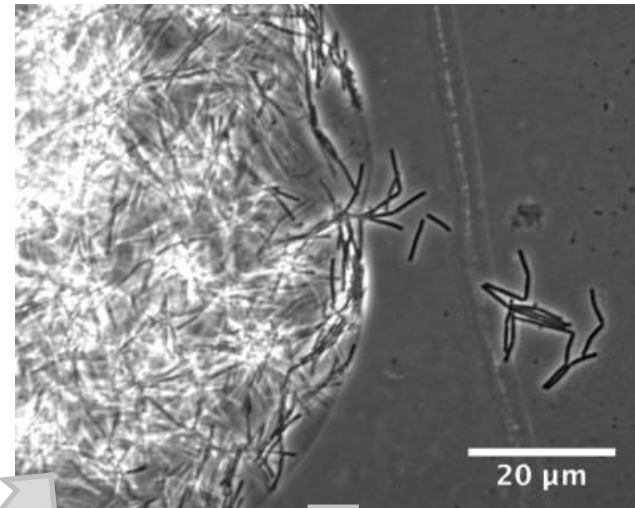
# Revival and growth of probiotic bacteria



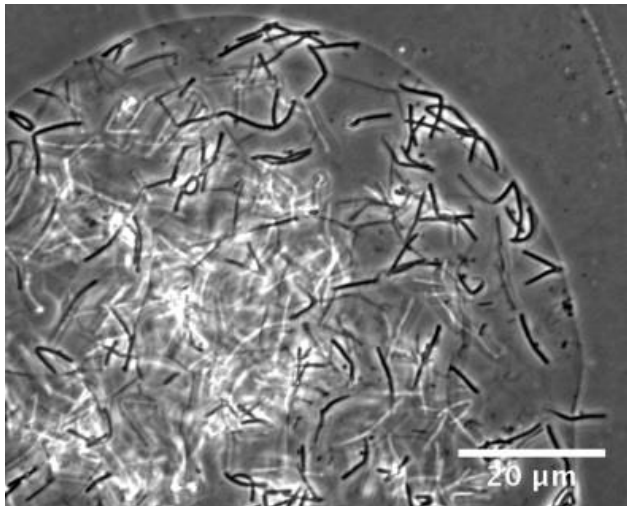
**Rehydration**



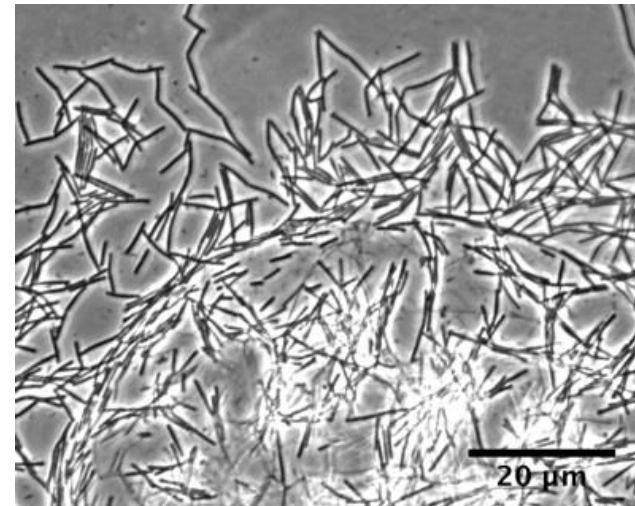
**Release of cells**



**Revival and buildup of cells**

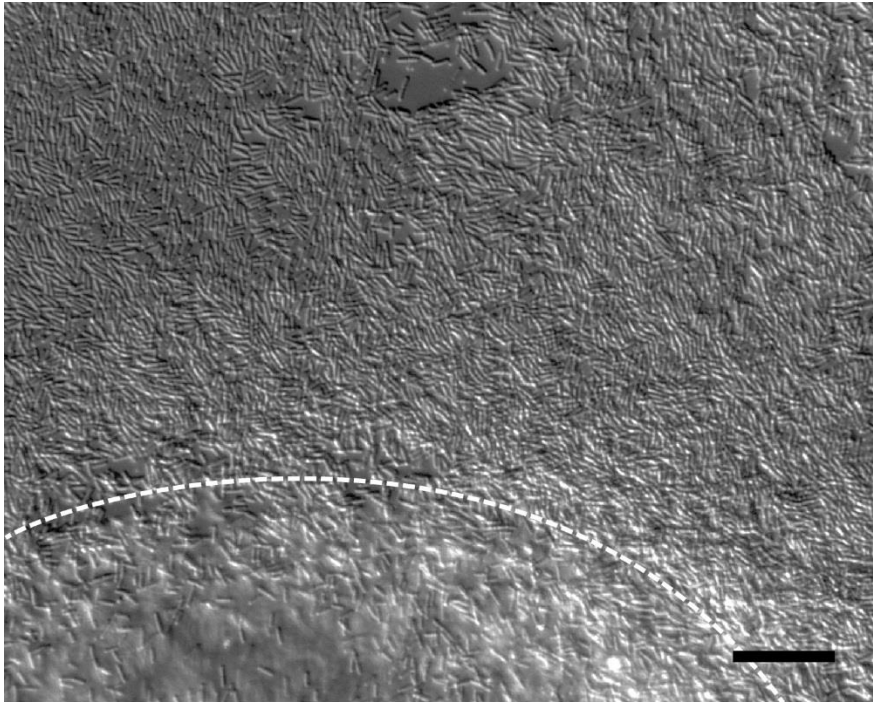


**Spreading over the surface**

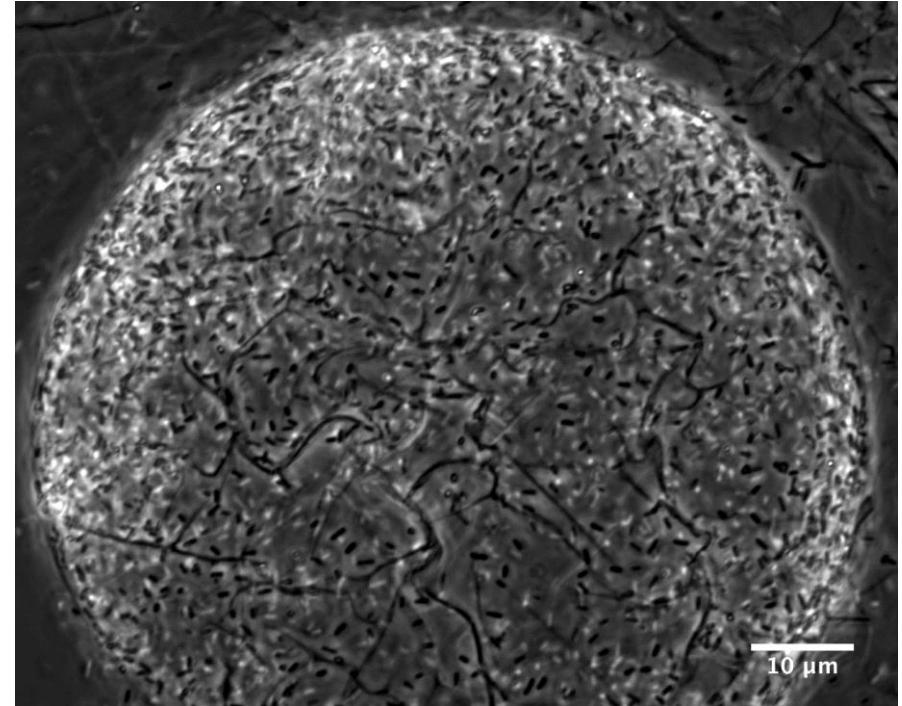




# Chitosan layer – entrapping the cells?



**Figure 3** Integrity of the alginate microcapsule is reduced due to disentanglement of alginate. Undefined surface of microcapsule marked with white dashed line.

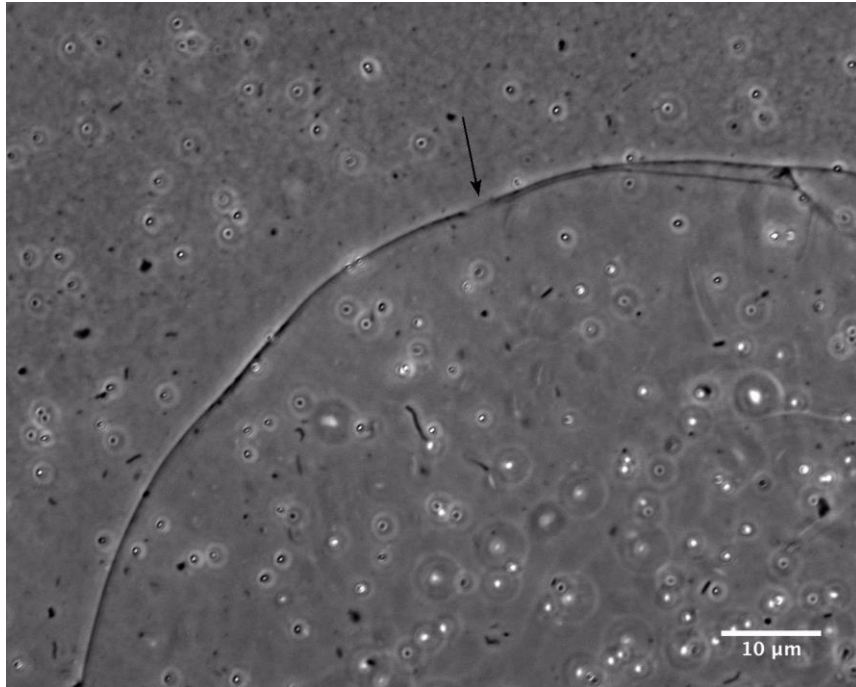


**Video 2** The chitosan-coated alginate microcapsule with the build up of growing and dividing cells. The intact chitosan coating prevents the cells to spread out of the microcapsule.

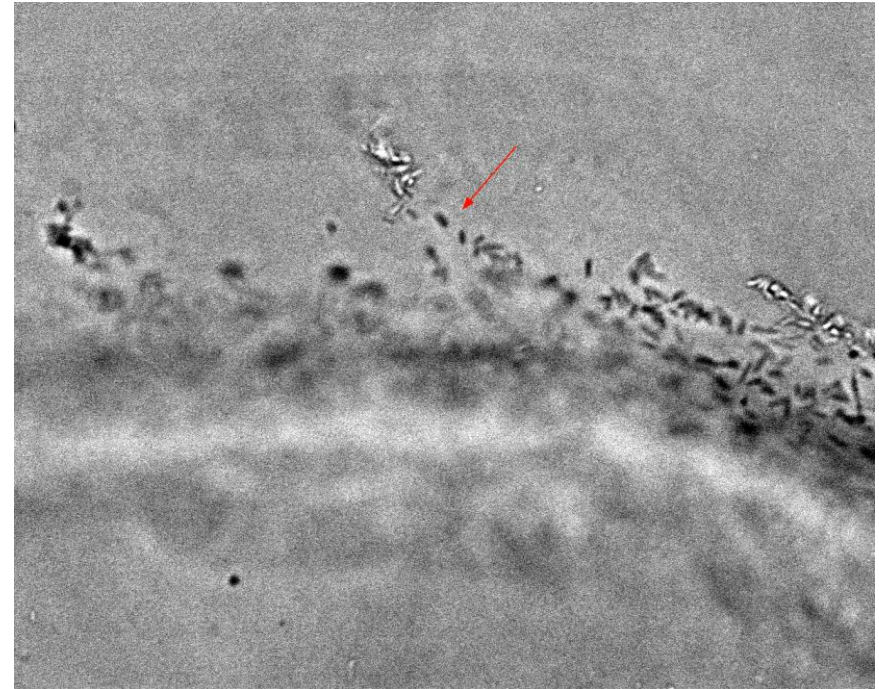




# Escaping the microcapsule chitosan layer



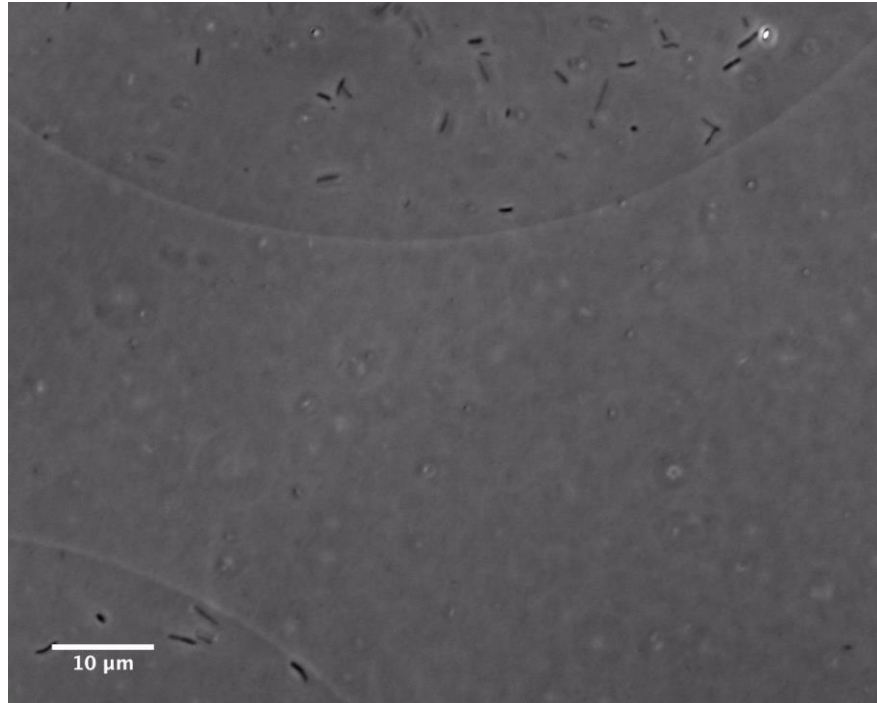
**Video 3** After the microcapsules are applied to the surface, the spores are released through an opening in the chitosan-coating (arrow) before the cells are revived and start dividing.



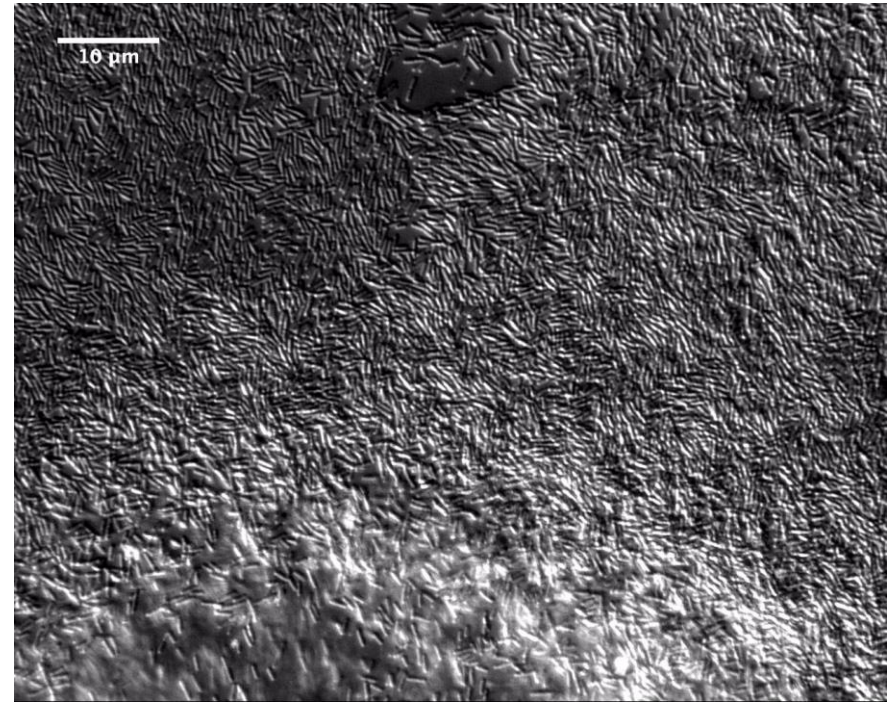
**Video 4** Vegetative cells are firstly revived and form microcolonies that are escaping microcapsule at cracked chitosan layer.



# Bacteria overgrowth



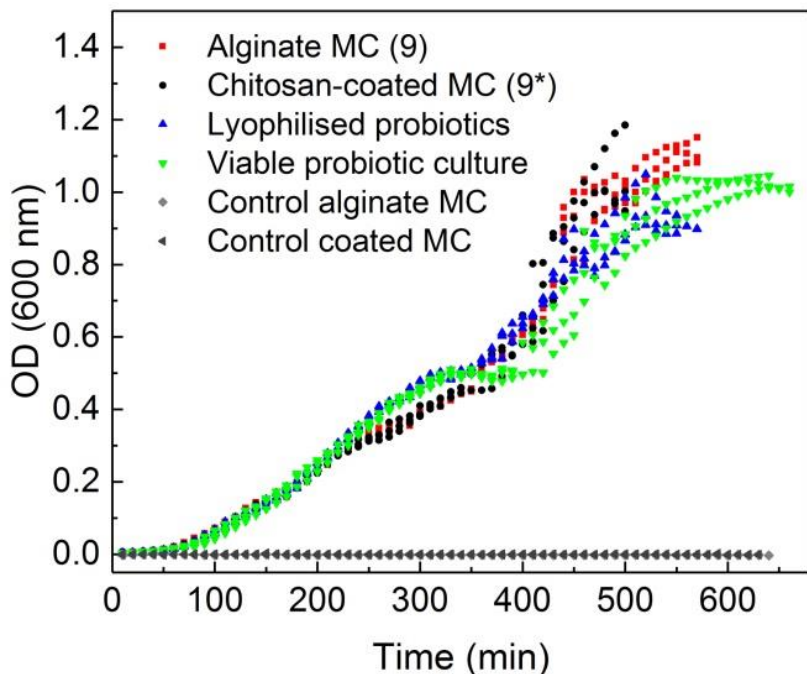
**Video 5** Growing, dividing and motile bacterial cells are spread over the surface of the agar medium to occupy the empty space between the microcapsules.



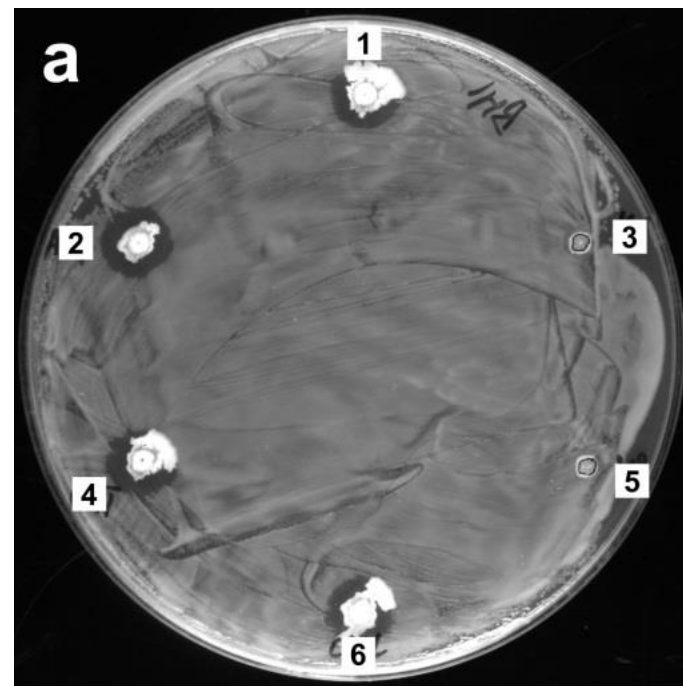
**Video 6** The integrity of the alginate microcapsule is reduced and the bacterial cells overgrow the microcapsule and the surface surrounding it.



# Growth kinetics and antibacterial activity



**Figure 4** The growth kinetics of bacterial cells originating from microcapsules, lyophilized cells (positive control) and empty microcapsules (negative control).



**Figure 5** Modified antibiogram showing antimicrobial activity of strain 4.1.Z cells encapsulated in the microcapsules.

- 1 – viable bacterial culture (positive control),
- 2 – alginate microcapsules,
- 3 – empty alginate microcapsules (neg. control),
- 4 – chitosan-coated alginate microcapsules,
- 5 – empty chitosan-coated alginate microcapsules (neg. control),
- 6 – lyophilized cells (positive control).



# Key message

Microcapsules based on alginate matrix and chitosan polyelectrolyte coating are promising probiotic delivery system, that enable:

- Adequate probiotic entrapment
- Viability during encapsulation process
- Prolonged survival
- Enhanced bioadhesion potential
- Modified probiotic release
- Antimicrobial activity



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  - Program **P1-0189** Pharmaceutical technology: from drug delivery systems to therapeutic outcomes of medicines in children and elderly
  - Project **J4-7640** Electrostatic immobilisation of bacterial cells and effects on their physiology
  - Project **J1-6746** Intra-pocket-targeted nanomedicines for treatment of periodontal disease



**THANK YOU!**



# Literature

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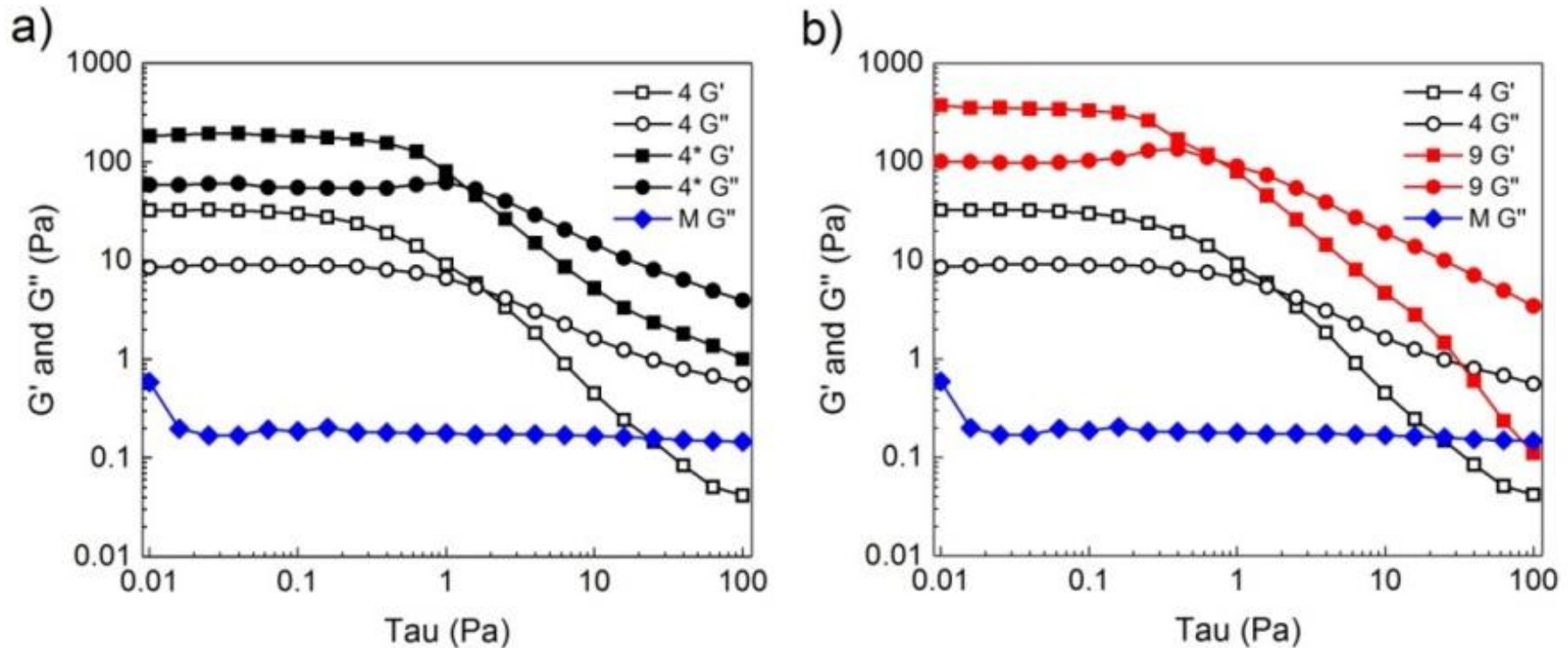
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# BACK-UP slides





# Viscosimetric assay of bioadhesion



**Figure 2** Storage ( $G'$ ) and loss ( $G''$ ) moduli measured in stress sweep mode showing effect of chitosan coating (formulations 4 and 4\*) (a) and of increased alginate concentration (formulations 4 and 9) (b); both graphs have data of  $G''$  for mucin dispersion (M) as a comparison.