Formation of Biofilm-Like Layer of Probiotic Bacteria in Microgel Using Flagellar Display for Oral Delivery of Cancer Targeting Peptide

Eric Boheen, Kang Wu, Linqing Li
University of New Hampshire Bioengineering/ Chemical Engineering

Abstract

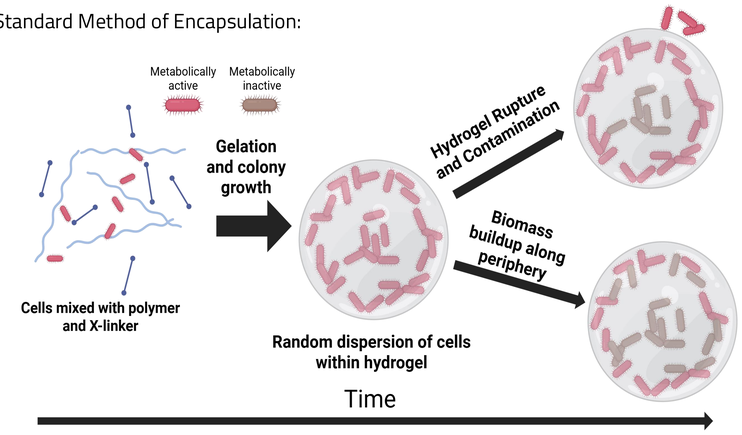
Recently engineered bacteria have reemerged as promising avenues for drug delivery and immunotherapy due to the development and advancements of novel peptide-based therapeutics. However, hurdles and critical challenges must be addressed before these modified bacteria can reach clinical setting applications. While non-invasive oral administration is preferred, it presents significant obstacles, including bacterial survival through the harsh gastrointestinal tract environment, prolonged viability during storage, and effective diffusion of therapeutic molecules to targeted cells.

Encapsulating bacteria within hydrogel matrices has emerged as a strategy to shield probiotic cells from the acidic and proteolytic conditions of the gastrointestinal tract while providing a suitable microenvironment for cell viability and growth. However, conventional hydrogel encapsulation often leads to a reduced bacterial population due to biomass accumulation at the hydrogel periphery, inhibiting nutrient diffusion to the gel interior and causing extensive cell death. To overcome these limitations, we propose an alternative probiotic encapsulation strategy that achieves spatial control of bacterial colony and growth using a layered microgel formulation. In this innovative system, dextran methacrylate microgel cores are produced independently using a microfluidic device, followed by chemical attachment of engineered probiotic bacteria via a thiol Michael-type addition reaction. Engineered E. coli cells displaying thiol-containing amino acids on modified flagellin proteins will be chemically conjugated onto the surface of dextran methacrylate cores via reaction with available methacrylate residues, followed by an alginate shell encapsulating the bacteria as a 3D monolayer. This design ensures the formation of a single bacterial layer at the interface between the microgel core and alginate shell, facilitating controlled diffusion of molecules and drastically enhancing cell viability and colony clustering by eliminating randomly dispersed bacterial colonies.

Introduction

Cell Encapsulation is the process by which cells are encased in a hydrogel matrix that allows for the diffusion of essential nutrients in and out of the gel. The matrix allows for the creation of a more suitable growth environment with modifiable mechanical properties. Most methods of encapsulation involve placing the cells in the gel reaction mixture and having the solid matrix form around the cells, however, this leads to a spatially heterogenous dispersion of cells within the gel. This in turn leads to uneven access to nutrients, resulting in differential growth.

Standard Method of Encapsulation:



Proposed Encapsulation Method:

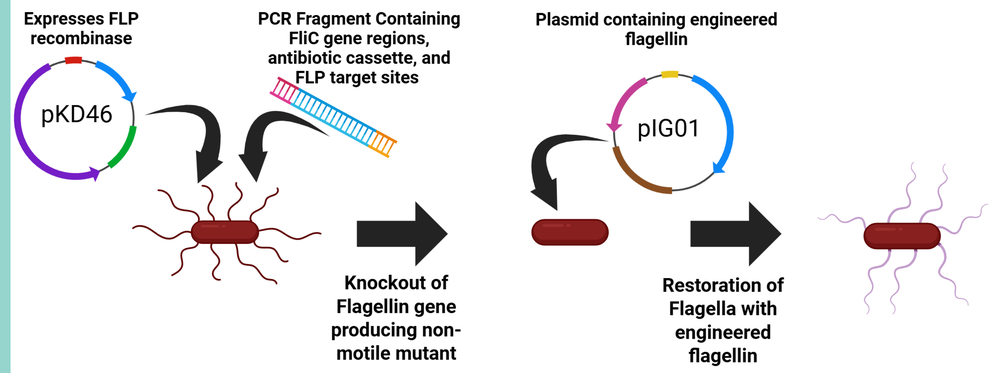
- Engineered Flagella enable the chemical binding of cells to the microgel surface
- A core-shell microgel is synthesized with bacteria at the core-shell interface
- Bacteria form a spatially uniform layer with even nutrient diffusion

Objectives

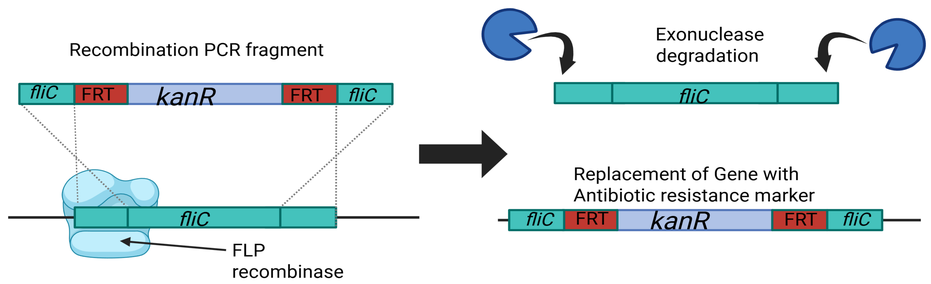
- Produce microgel beads with engineered bacteria capable of long-term storage at room temperature
- Develop and adaptable platform for oral delivery of drugs utilizing recombinant bacteria

Methods

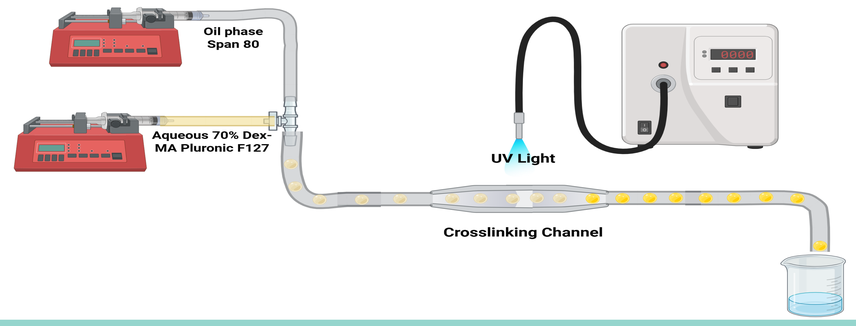
Engineered probiotics:
The probiotic bacteria E. coli Nissle 1917 was engineered via phage lambda red recombinase knockout of the *fliC* gene that expresses flagellin and restoration with a recombinant form of the gene.



FLP Recombinase Mediated Knockout of *fliC*:

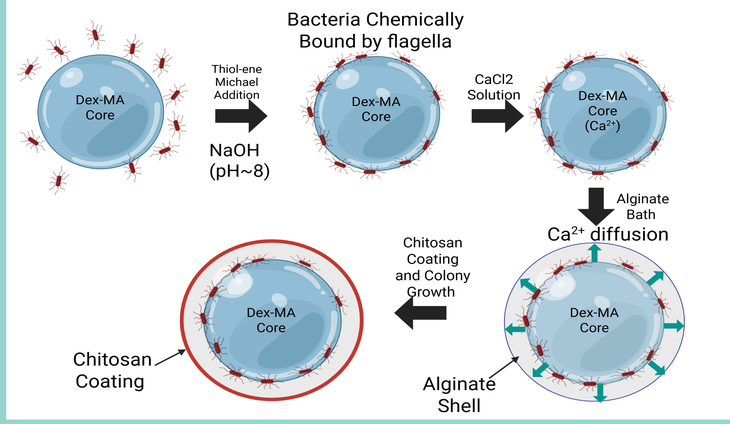


Microfluidic production of UV crosslinked hydrogel cores:



Methods continued

Spatially Controlled Probiotic Encapsulation Method Core-Shell:



Future Plans

Project Plans

- Small molecule diffusion test utilizing arabinose-induced p28 anti-cancer peptide expression
- Survival of probiotics in simulated gastric acid
- Drug Delivery efficacy test utilizing HT-29 Colorectal cancer cells
- Long-term cell viability assay in storage media

Applications:

- Delivery of Peptide-Based drugs to the gastrointestinal tract with tunable expression and release
- Co-delivery of therapeutic bacteria with drug-loaded
- Long-term storage of probiotics

Acknowledgements

Project funding provided by the Hamel Center for Undergraduate Research and the UNH CEPS Dean's office.

The project is currently being carried out by the UNH iGEM Team, CEPS student organization, and has multiple student and faculty contributors.

Special thanks to Dr. Nivedita Gupta for assistance in the microfluidic system design