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# Microencapsulation of Intestinal Bacteria: RGD Functionalization for Improved Cell Activity

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ABSTRACT

The human gut microbiota plays an important role in human health; however, our understanding of the bacteria which make up the microbiota remains limited. The gaps in current knowledge can be attributed to the difficulties in culturing a major part of this diverse community using conventional *in vitro* techniques. The lack or absence of cell-to-cell communication and the inability to accurately replicate optimal growth conditions and nutrient requirements are some of the key limitations apparent in standard culture. Additionally, current genomic techniques struggle to reveal bacterial physiology and function, as 16S rRNA and shotgun sequencing often miss crucial microbial roles and are affected by host DNA contamination. To address this challenge, we propose an innovative approach to support the growth of difficult-to-culture bacteria by employing droplet-based microfluidics. Microfluidics techniques have shown promise in human gut microbiota, research, but the role of microencapsulation on bacteria enrichment has yet to be explored. Microencapsulation has been explored for probiotic formulation and delivery, but no studies have attempted to harness the benefits of microencapsulation to promote viability and increase biomass of difficult-to-culture gut bacteria.

This study explores the use of four-arm poly(ethylene glycol) maleimide (PEG4MAL), a synthetic, non-digestible, and biocompatible material, for the microencapsulation of highly sensitive anaerobic bacteria. PEG4MAL is functionalized with 0.8 mM of RGD, and crosslinking is achieved through a rapid thiol-based reaction with 20mM dithiothreitol (DTT). Encapsulation is performed using a 70 µm junction microfluidic chip for droplet generation with syringe pump driven flow. Encapsulated Escherichia coli and *Akkermansia muciniphila* are fluorescently imaged after staining with a Live Dead Assay.

We have demonstrated that single-cell isolation can be achieved through microbead production, which will enable the isolation of species for community-based culture. It was observed that cell behavior can be influenced by manipulating the concentration of PEG4MAL, with increased concentrations resulting in higher cell motility. Further modification of PEG4MAL through functionalization with arginylglycylaspartic acid (RGD) led to enhanced cell density within the microbeads due to peptide-cell interactions. By employing this approach, we achieved high cell viability and colony formation within the microbeads of the difficult-to-culture *Akkermansia muciniphila*. This increased aggregation facilitates the capture and analysis of a broader range of bacterial species, including those that are considered ‘unculturable’.

This work opens the door to *in situ* cultivation, allowing researchers to better characterize the human gut microbiota composition and its complex interactions with human health and disease.