MICROFLUIDIC MANUFACTURING OF LIPOSOMAL ADJUVANTS

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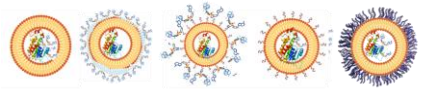
Introduction

Helicobacter pylori (H. pylori) infection remains a global health concern, and effective mucosal vaccines are still lacking. Our research aims to develop cationic liposomes capable of co-delivering an H. pylori antigen (H59a) to antigen-presenting cells (APCs) in the gastrointestinal tract. Cationic liposomes, composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and dimethyldioctadecylammonium (bromide salt) (DDAB), have demonstrated stability and compatibility with the vaccine platform. In addition, as an immunostimulatory adjuvant, cyclic di-adenosine monophosphate (CDA) provided by the Helmholtz Centre for Infection Research has shown potential to be a promising adjuvant in vaccine development. To achieve co-delivering an H. pylori antigen and CDA to APCs, we employed microfluidic technology, which offers precise control over formulation parameters and enables the production of liposomes with uniform and reproducible characteristics.
Based on this formulation, we also investigated both the composition and surface chemistry of the liposomes, as well as the location of CDA within the system, to explore the potential of various cationic liposomal platforms for mucosal vaccine development. The novel cationic liposomal formulations using microfluidics technology were successfully developed.

Method and Result

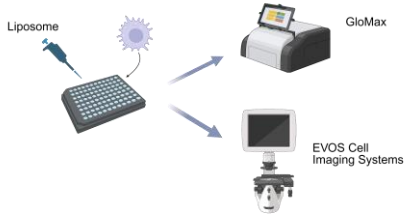
1 Formulation Production

Microfluidics technology | Dynamic light scattering (DLS) | Fourier Transform Infrared (FTIR) spectroscopy



Cyclic di-AMP and protein-loaded liposomes	Cyclic di-AMP-coated liposomes loaded with protein	Cyclic di-AMP-loaded PEGylated liposomes with protein	PEGylated liposomes loaded with cyclic di-AMP and protein	Mucoadhesive-coated liposomes loaded with cyclic di-AMP and protein

2 In-vitro Study



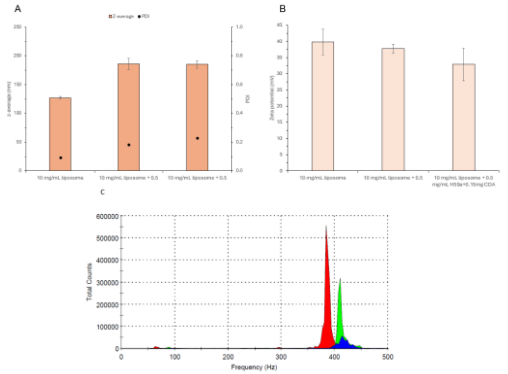


Figure 1: **The comparison of physicochemical properties of empty liposomes and liposomes with H59a and CDA**. A) Particle size (d.nm; represented by the bars) and PDI (represented by the discrete points) and B) zeta potential (mV; represented by the bars) measured after 24 hours of incubation at 4 ° C. C) Comparison of the frequency (Hz) shift of liposome with H59a and CDA (represented by the red colour), liposome with H59a (represented by the green colour), and liposome s(represented by the blue colour). Results represent mean ± SD, n=3 of independent batches.

	Zeta potential (mV)	SD
Formulation 1	41.2	1.4
Formulation 2	39.7	1.3
Formulation 3	38.4	1.24
Formulation 4	41.2	0.902
Formulation 5	12.1	1.91
Formulation 6	22.1	0.451
Formulation 7	44.9	1.57
Formulation 8	8.86	0.413
Formulation 9 A	35.8	0.764
Formulation 9 B	40.9	1.9
Formulation 10 A	42.8	2.08
Formulation 11 A	34	1.83
Formulation 11 B	38.3	1.21

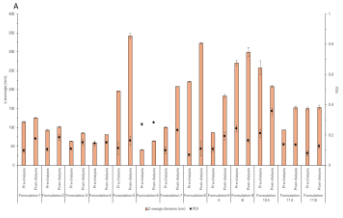


Figure 2: **The comparison of physicochemical properties of different liposomal formulations**. A) Particle size (d.nm; represented by the bars) and PDI (represented by the discrete points) of 13 liposomal formulations. The table shows the zetapotential of 13 liposomal formulations. Results represent mean ± SD, n=3 of independent batches.

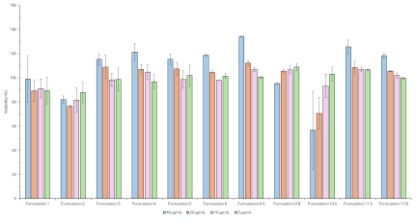


Figure 3: **The cell viability of different liposomal formulations**. Cell viability (%) of Raw 264.7 cells treated with 11 liposomal formulations at OVA concentrations of 40, 20, and 10 µg/mL for 24 hours. Viability is represented by bars. Data are shown as mean ± SD (n = 3 independent batches).

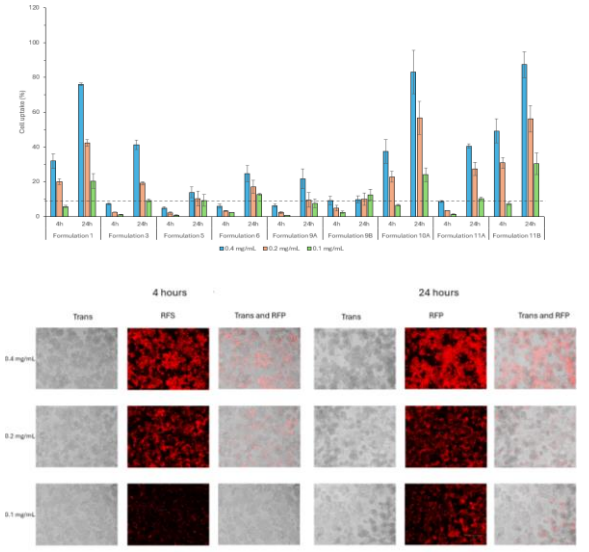


Figure 4: **Cell uptake of different liposomal formulations.** A) Quantification of cell uptake (%) for nine liposomal formulations in Raw 264.7 cells after 24-hour incubation at 0.4, 0.2, and 0.1 mg/mL OVA concentrations. Uptake values above a dotted line were considered valid. B) Fluorescence microscopy images of the formulation with the highest uptake, captured at 4-hour and 24-hour time points. Data represent mean ± SD (n = 3 independent batches).

Conclusion

- Cationic liposomes produced via microfluidics successfully co-encapsulated the H59a antigen and CDA adjuvant, demonstrating efficient formulation control and reproducibility.
- A total of 11 novel formulations were developed by varying the cationic lipid, cholesterol content, and helper lipid composition.
- All formulations maintained cell viability above 80%, indicating good biocompatibility with Raw 264.7 cells.
- 7 formulations showed effective cellular uptake, highlighting their potential for mucosal vaccine delivery.

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