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***In vitro* interaction between macrophages and cubosomes for oral vaccine delivery imaged by coherent anti-Stokes Raman scattering (CARS) and automated microscopy**

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Learning objectives:

1. Explain the advantages of using CARS and automated microscopy for investigating particle interaction with macrophages
2. Describe what information can be obtained from the CARS images
3. Discuss if the cubosomes show promise as a vaccine formulation based on the CARS and automated microscopy results

INTRODUCTION: Oral vaccination is very advantageous compared to vaccination administered by injection, but to succeed with oral vaccination, a combination of a subunit antigen, an adjuvant and a particulate system is essential¹. *In vivo* studies are normally performed to test the effect of a developed vaccine formulation, but it will be very beneficial if the efficacy can be tested *in vitro* by e.g. studying the interaction between a vaccine formulation and immune cells. The aim of this work was to visualize the interactions between the lipid particulates, cubosomes (with ovalbumin and Quil-A) and macrophages using CARS and automated microscopy (using an oCelloScope).

METHODS: Cubosomes were prepared by dissolving 5.33 w/v% of Dimodan® (glycerol monooleate) in ethanol and mixing it with an aqueous solution of the stabilizer, dextran, the model antigen, ovalbumin and the adjuvant, Quil-A in concentrations of 2.63, 0.52, 0.035 mg/mL, respectively. The solution was spray dried on a Büchi mini spray dryer.

RAW 264.7 were seeded in a glass well plate with 100.000 cells per well in 1 mL of cell medium, and incubated overnight at 37°C and 5% CO₂. The following day, the seeded cells were incubated for 8 h with 0.1 mg/mL cubosome formulation.

For CARS studies, after 8 h of incubation, the cells were stained with cellMask orange stain followed by fixing the cells. Subsequently, the cells were imaged using two-photon excited fluorescence and CARS microscopy (Leica TCS SP8) with stokes laser beam at 1064.5 nm and pump laser at 816 nm.

For the automated microscopy study, the incubation of RAW 264.7 cells and cubosomes were followed live for 8 h at 37°C and 5 % CO₂ on a oCelloScope (Biosense Solutions) taking two images in each well every 30 min.

RESULTS: After 8 h of incubation, it was found with two-photon excited fluorescence and CARS microscopy that the cubosomes interacted with the macrophages (Fig. 1). With CARS spectra, it was possible to differentiate between cells and particulates and thereby, observe where the cubosomes were located in

the macrophages. For the automated microscopy, it was seen that the cells increased in area over time when incubated with cubosomes with ovalbumin and Quil-A. This was significantly different to the cell area when the RAW cells were incubated with medium or ovalbumin solution (controls).

CONCLUSIONS: CARS and automated microscopy can be used to study the interaction between lipid particles as cubosomes and macrophages.

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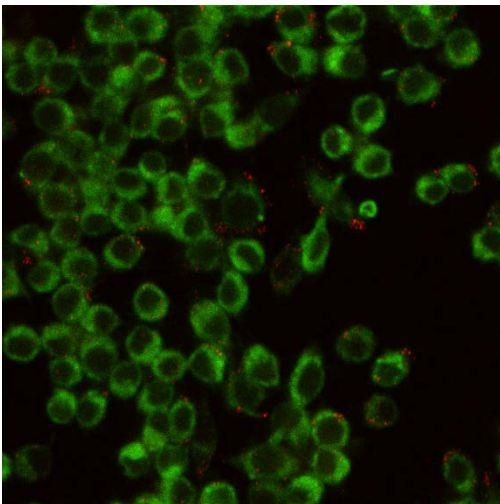


Fig. 1: Example of a CARS image of stained RAW 264.7 cells (in green) incubated for 8 h with cubosomes with ovalbumin and Quil-A (red dots).