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Microcontainers for oral vaccine delivery

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INTRODUCTION
Most vaccines are administered by injection, but the oral route can be beneficial for vaccine administration due to the ease of administration and the potential for mass vaccination without the requirement of trained personnel. For vaccine formulations, peptides are often utilised as the antigen (so called subunit vaccines), although these are often poorly immunogenic. To overcome this, the antigen is incorporated in a particulate system, often together with adjuvants. An example of such particulate system is cubosomes. Cubosomes are highly twisted, continuous lipid bilayers with two congruent, non-intersecting water channels providing both hydrophilic and hydrophobic domains and a large surface area for association of antigens [Rizwan et al., 2011]. For delivering vaccine formulations by the oral route, micro fabricated drug delivery devices can be used. Of these micro devices, microcontainers are suggested as especially promising [Nielsen et al., 2016]. Microcontainers are polymeric, cylindrical devices in the micrometer size range (Figure 1). A potential advantage of microcontainers is that these devices allow for unidirectional release, as only one side of the microcontainers is open. Moreover, microcontainers with coatings can protect the formulation through the stomach and allow for controlled release in the intestine. In addition, microcontainers have been observed to interact with the intestinal mucus layer resulting in prolonged and increased absorption of poorly soluble drugs compared to controls without microcontainers [Nielsen et al., 2016], [Mazzoni et al., 2017].

Figure 1: SEM image of a microcontainer

The purpose of these studies was to prepare glycerol monooleate-based cubosomes carrying the model antigen ovalbumin and the adjuvant Quil-A using spray drying as preparation method as well as to in vitro characterise these particles. Furthermore, the cubosome formulation was loaded into microcontainers to be further tested for the potential application in oral vaccine delivery.

EXPERIMENTAL
SU-8 microcontainers were fabricated using two steps of photolithography [Nielsen et al., 2012]. Cubosomes consisted of the commercial glyceryl monooleate, Dimodan®, containing ovalbumin (OVA) and Quil-A, and were stabilized with a dextran shell. Dimodan was dissolved in ethanol (5.33 w/v%) and mixed with an aqueous solution of dextran, OVA and Quil-A (2.63, 0.52, 0.035 mg/mL, respectively). After mixing, the solution was spray dried on a Büchi mini spray dryer (Figure 2).

Figure 2: Schematic of the preparation of cubosomes produced by spray drying.

Cryo-TEM was used to verify the cubic particle morphology after reconstitution of the spray dried cubosomes in aqueous dispersion. Moreover, the size and zeta potential of the particles in aqueous suspension were measured by dynamic light scattering. The amount of loaded OVA and release of OVA from the cubosomes in PBS at pH 7.3 was measured by fluorescence. After in vitro characterisation, the cubosome powder was loaded into SU-8 microcontainers either using a powder embossing method (Figure 3) or by manually filling the cubosomes into the microcontainers. The filled
microcontainers were checked using scanning electron microscope (SEM) and x-ray microtomography.

![Figure 3: Schematic of the powder embossing method for loading the microcontainers with the cubosome powder.](image)

The SAXS/WAXS beamline at the Australian Synchrotron, Clayton, Australia was used to determine the internal structure of the spray dried particles. The microcontainers were manually filled with cubosomes (with OVA) and small-angle X-ray scattering (SAXS) was performed on the dry particles and when hydrated in buffer at pH 6.8. Furthermore, following vaccine-loading, a lid of the pH sensitive polymer Eudragit L100-55 was deposited on the cavity of the microcontainers by a spray coating system for protection of the vaccine formulation through the stomach.

**RESULTS**

SU-8 microcontainers had an inner diameter of 220 µm and a cavity depth of 270 µm (Figure 1).

The spray drying process produced cubosomes as verified by cryo-TEM (Figure 4) and SAXS. The particle size of the cubosomes in suspension was 257±8 nm and the zeta potential was -18±0.6 mV. Approximately 106 µg of OVA was present in 1 mg of powder, and the release of OVA was fastest initially and gradually slowed down until 100 % was released within 24 h.

![Figure 4: Cryo-TEM image of a cubosomes after redispersing the cubosomes powder in aqueous medium.](image)

After characterization, the powder of cubosomes was loaded into the microcontainers resulting in cubosome-filled microcontainers shown in Figure 5.

![Figure 5: Loading of the microcontainers with cubosomes. a): SEM image of the loaded microcontainers, and b) & c): x-ray microtomography images of the loaded microcontainers.](image)

In addition, microcontainers were also manually loaded with cubosomes containing OVA and SAXS analyses revealed that the ‘dry’ particles possessed an internal ordered lipid structure (lamellar and inverse micellar phase) by virtue of a small amount of residual water. After hydration in buffer at pH 6.8, the particles formed a hexagonal inverse cubic phase, indicating that cubosomes were formed when released from microcontainers (Figure 6).

![Figure 6: 2D SAXS patterns were collected from cubosomes confined in microcontainers and followed while the cubosomes were released from the microcontainers. The cubosome-filled microcontainers were enclosed in a glass capillary during hydration for up to 80 min.](image)

The Eudragit lid on the cavity of the microcontainers protected the vaccine formulation inside the microcontainers through the pH of the stomach and provided a release in the pH of the intestine verified by SEM.

**CONCLUSION**

A dry powder of cubosomes loaded with OVA and Quil-A was produced by spray drying. After characterisation, the powder was loaded into microcontainers, and SAXS analyses indicated that cubosomes were released from the microcontainers.

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