2012

Matrix for Chemical IT
(MATCHIT)

Deliverable
Report
D2.3

Grant Agreement No. 249032

D2.3) Fission process and a subsequent tag-addressing round: Implementation of the fission process (without loss of content) and a subsequent successful addressing round. [month 36] **Delivered in month 36**

This final deliverable for WP2 is to show a sequential operation performed on chemtainers using the ssDNA addressing system: 1) Implementation of the fission process (without loss of content) and the 2) subsequent successful addressing round. This goal has been achieved with the vesicle system.

Vesicle-vesicle chemtainer system

The entire experimental design is depicted in Figure 1. SDUa first produced vesicles by our standard oil-in-water emulsion technique to produce GUVs with red fluorescent cargo (Atto 565-Biotin). This vesicle population contains **an 'open' address** of biotin-conjugated lipid. The GUVs were then divided by a standard extrusion **fission process** through polycarbonate membrane with 12 micron pore size. After fission, the smaller vesicles were **'readdressed'** with a ssDNA through the open biotin linkage and blue streptavidin 350. Confirmation of these steps is shown in the micrographs in Figure 1. In all panels g-k small red vesicles are seen with a blue membrane indicating that vesicle contents (red) were retained after the fission operation. The blue fluorescence from the streptavidin indicates that the post-fission addressing was successful. For further confirmation that the system is working as expected, the divided and addressed small vesicles were incubated with large vesicles labeled with green streptavidin 488. When the divided vesicle population was readdressed with ssDNA complementary to the green vesicles, specific association occurred (Figure 1h). When the divided vesicle population was readdressed with noncomplementary ssDNA as a control, no vesicle-vesicle association was detected (Figure 1k).

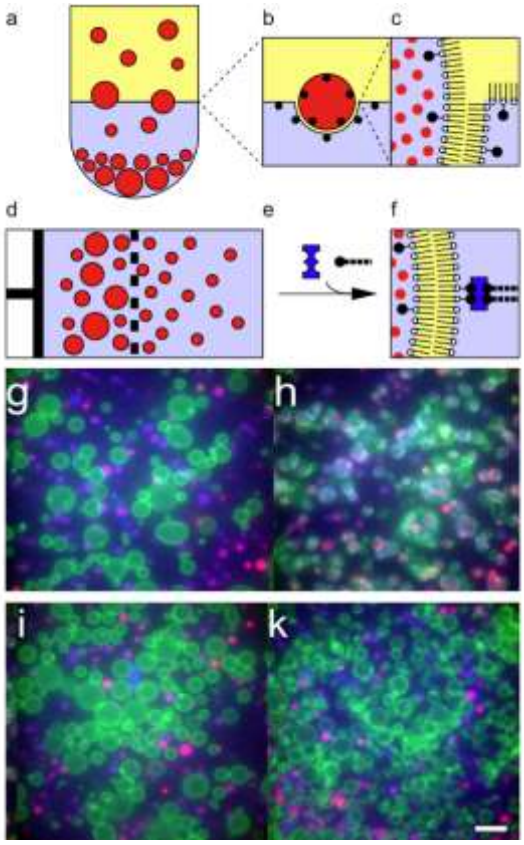

Figure 1. Scheme and micrographs detailing vesicle fission and subsequent addressing without loss of contents. a-c) initial vesicle preparation with biotin 'open' address and red fluorescent internal contents d) vesicle fission e,f) subsequent addressing with ssDNA through a biotin (black circle) and blue fluorescence labeled streptavidin linkages. g,h) micrographs of the self-assembly of divided (blue membrane and red contents) and non-extruded vesicles (green membrane) g) after merging the two populations and h) after 5h of incubation of the two vesicle populations with complementary ssDNA addresses, i,k) control micrographs: two vesicle populations are merged but the ssDNA on the vesicle surfaces is non-complementary. Scale bar: 10um.

We implemented the well-established vesicle extrusion procedure described above in a microfluidic platform with our partners at Osaka University. Instead of using acid-etched polycarbonate filters to perform extrusion, we designed successive rows of nanoscale slits into microscale channels all made of PDMS. We then used syringe pump driven flow to push GUVs through the small slits. This resulted in small vesicles passing through the slits, larger vesicles halting at the slits effectively blocking the slit, or larger vesicles passing through the slits and being physically extruded to a smaller diameter. The overall layout of

the microfluidic vesicle divider is shown in the accompanying figure 2A and the microscopic image taken from a movie of vesicle division through the microfliuidic slit is shown in 2B. Because the divided vesicles are still visible due to their higher refractive index due to encapsulated solute after division we know the contents have been retained.

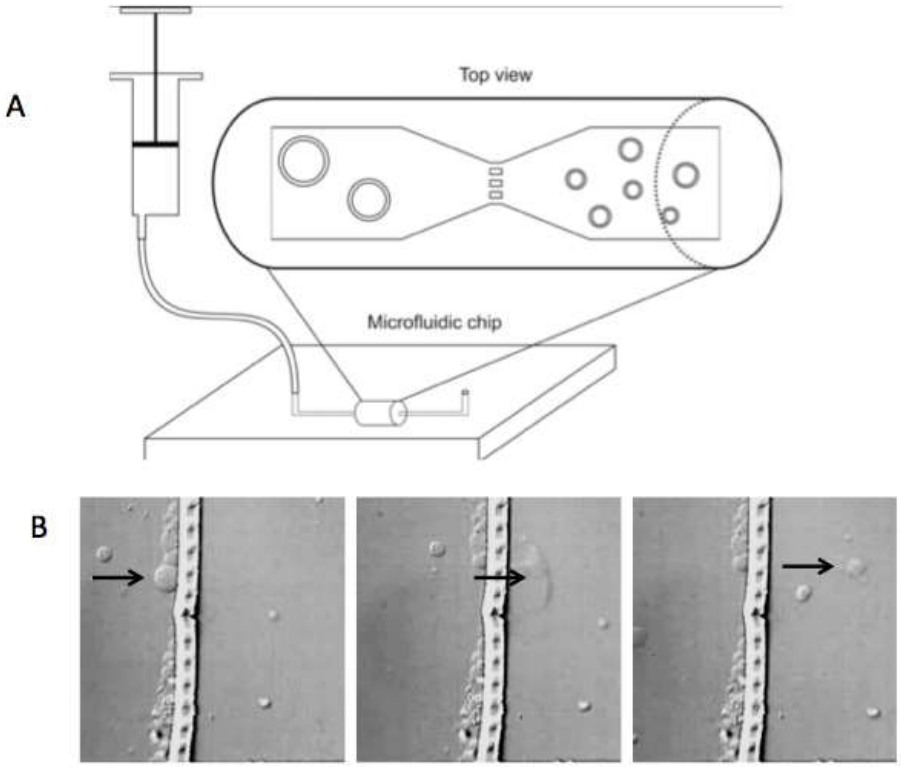

Figure 2. Vesicle division in a microfluidic platform. A) design of microfluidic vesicle division device. Concentric circles in the top view represent vesicles and the small rectangles delineate the small slit. The syringe pumps cause the constant flow of material from left to right. B) white light microscopy shows a large vesicle denoted by the arrow that then is passed through a slit, divides and becomes smaller. Time scale of images: 1 second total. Size of each frame: 100 x 100 microns.

This work has not yet been published and is therefore confidential. However we plan to include this data in a larger paper that details this system within a larger experimental context within the next year.

Droplet-droplet chemtainer system

SDUa made several attempts to merge the spontaneous fission of oil droplet chemtainers with the ssDNA addressing system. Initially we found two incompatibilities: the oil phase used to show DNA address specific droplet-droplet assembly was not compatible with the conditions for spontaneous droplet fission. Secondly, we found that the ssDNA anchoring system was not compatible with spontaneous droplet fission. The first incompatibility has been overcome but the second remains an obstacle. Therefore a complete oil droplet system that shows both DNA-directed droplet-droplet assembly (Hadorn et al, 2012) and spontaneous fission (Caschera et al, 2012) has not been achieved. We are currently pursuing several solutions to this incompatibility but these will not be completed within the time frame of MATCHIT. Therefore as a deliverable we are submitting the vesicle-vesicle system as described above since both assembly and division works well.

Hadorn M, Boenzli E, Sørensen KT, Fellermann H, Eggenberger Hotz P, Hanczyc MM. 2012a. Specific and Reversible DNA-directed Self-Assembly of Oil-in-Water Emulsion Droplets. PNAS, doi: 10.1073/pnas.1214386109.

Caschera F, Rasmussen S, Hanczyc MM. 2012. An Oil Droplet Division-Fusion Cycle, ChemPlusChem, DOI: 10.1002/cplu.201200275.