Cellular uptake and cell-to-cell transfer of polyelectrolyte microcapsules within a triple co-culture system representing parts of the respiratory tract

Dagmar A. Kuhn\textsuperscript{1*}, Raimo Hartmann\textsuperscript{2*}, Kleanthis Fytianos\textsuperscript{1}, Alke Petri-Fink\textsuperscript{1}, Barbara Rothen-Rutishauser\textsuperscript{1†}, Wolfgang J. Parak\textsuperscript{2,3†}

\textsuperscript{1} Adolphe Merkle Institute, Université de Fribourg, Fribourg, Switzerland
\textsuperscript{2} Department of Physics, Philipps Universität Marburg, Marburg, Germany
\textsuperscript{3} CIC Biomagune, San Sebastian, Spain
\* both authors contributed equally
\† corresponding authors: wolfgang.parak@physik.uni-marburg.de; barbara.rothen@unifr.ch

Supporting Information

<table>
<thead>
<tr>
<th>Type</th>
<th>Encapsulated cargo</th>
<th>Polyelectrolytes</th>
<th>(N_{bi})</th>
<th>Diameter/(\mu)m</th>
<th>Fig</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEMs for flow cytometry</td>
<td>FITC-Dextran 500kDa</td>
<td>PSS PAH</td>
<td>4</td>
<td>3.3 ± 0.3</td>
<td>A</td>
</tr>
<tr>
<td>PEMs for release/transport experiments</td>
<td>BODIPY-labeled DQ-OVA</td>
<td>DextS PLArg</td>
<td>4</td>
<td>4.7 ± 0.4</td>
<td>B</td>
</tr>
</tbody>
</table>

Table SI-1: Characteristics of polyelectrolyte multilayer capsules (PEMs) used for the experiments (Figure ). \(N_{bi}\) describes the number of bilayers of which the capsule shell is composed. The size distributions (mean value ± standard deviation, capsules were assumed to be circular) were derived computer-aided from confocal fluorescence micrographs, such as the ones presented in Figure . More than 800 capsules were analyzed per sample. Details about the capsule segmentation procedure can be found in the supporting information of Hartmann et al\textsuperscript{1}.

Figure SI-1: Fluorescence micrographs (left: green channel, middle: brightfield image, right: composition) of polyelectrolyte multilayer capsules used for the experiments. The labels A-C correspond to the samples listed in Table (last column). The scale bar corresponds to 20 µm.

Figure SI-2: Biodegradable PEMs engulfed by MDMs at 1 hour of exposure. Cells are shown in differential interference contrast (DIC) and PEMs are shown in green. The scale bar corresponds to 20 µm.
Figure SI-3: Flow cytometry gating strategy for the bi-cultures. (A) Identification of the different cell populations in MDMs-MDDCs bi-cultures. The cells were stained with specific surface markers (MDDCs: CD1c-Pacific Blue; MDMs: CD14-Brilliant Violet). Initially, unspecific gating was applied (FSC vs. SSC) in order to exclude cellular debris. Afterwards, the populations were identified according to the fluorescence intensity of each specific marker. Finally, the FITC intensity was utilized to determine capsule uptake. Each experiment was repeated three times (n=3) and cells from different cell cultures were used. Capsule-: samples without capsules; Capsule+: samples treated with capsules. (B) % Frequencies of the different cell populations in the bi-cultures; light grey: unstained control; dark grey: stained sample. The entire procedure was performed at 4°C. The numbers represent the cells expressing the markers. $I_{CD1c}$-Pacific Blue and $I_{CD14}$-Brilliant Violet correspond to the fluorescence intensity of the MDDC and MDM marker, respectively.