Targeting the Immune System with Biodegradable Nano Engineered Polymeric Microcapsules

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Vaccines that can elicit strong T-cell responses are undoubtedly one of the major challenges for medicine today. [1] For this purpose, dendritic cells (DCs) have to internalize antigen, process them into peptide fragments and present them to T-cells. Whereas soluble antigen largely fails to induce potent cellular immune responses, formulation of antigen into microparticles has emerged as an attractive alternative. Here, we demonstrate efficient *in vitro* and *in vivo* antigen delivery to DCs using biodegradable polyelectrolyte capsules as antigen carrier.

Figure 1 schematically shows the encapsulation procedure of antigen into hollow polyelectrolyte capsules. [2,3] In a first step, antigen loaded $CaCO_3$ microparticles (3 µm diameter) are fabricated by co-precipitation of $CaCl_2$ and Na_2CO_3 in the presence ovalbumin (OVA) as model antigen. Subsequently these $CaCO_3$ microparticles are coated (2 bilayers) by sequential deposition of dextran sulfate and poly-L-arginine using electrostatic interaction as driving force. Finally hollow polyelectrolyte capsules are obtained after dissolution of the $CaCO_3$ core templates in aqueous EDTA medium.

As demonstrated by transmission electron microscopy (Figure 2A) and confocal microscopy (figure 2B), polyelectrolyte capsules are efficiently taken up by DCs and both the capsule membrane as well as the encapsulated antigen becomes readily processed. [4,5] Antigen presentation to T-cells was assessed by incubating DCs with OVA loaded capsules followed by co-culturing with respectively OT-I and OT-II cells (Figure 2C). OT-I and OT-II cells are transgenic CD8, respectively CD4 T cells that specifically recognizes the OVA CD8 peptide, respectively CD4 peptide. Compared to soluble antigen a dramatic increase in T-cell presentation is observed. Especially cross-presentation to CD8 T-cells, which are crucial to induce cellular immune responses, is strongly promoted. [4]

In vivo studies show mild tissue reactions [6,7] upon subcutaneous injection [5 while potent humoral and cellular immune responses [8] are induced which show protective immunity against viral infection as well as cancer. Moreover in recent studies we have also demonstrated an easy strategy – involving main stream pharmaceutical technology – to scale the production of polyelectrolyte microcapsules using a one-step procedure which encapsulates antigen with extremely high yields while barely hampering its biological activity. [9]

References

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Figures

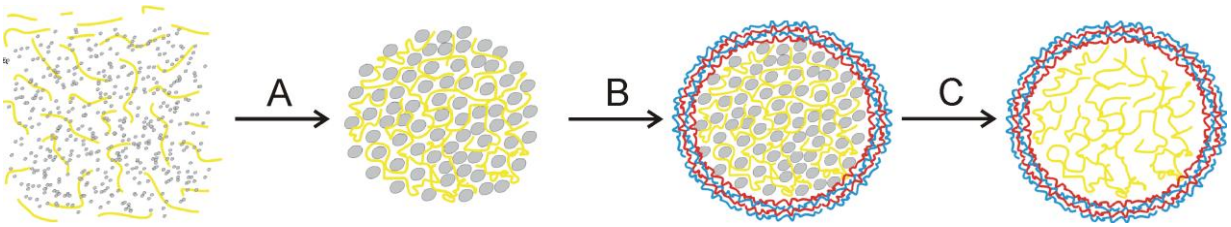

Figure 1. Polyelectrolyte microcapsule synthesis. (A) Antigen (yellow) is mixed with $CaCl_2$ and Na_2CO_3, resulting in the generation of macromolecule-filled $CaCO_3$ microparticles (gray), which are (B) subsequently coated with alternating layers of dextran sulfate and poly-L-arginine (red, blue). (C) Dissolution of the $CaCO_3$ core by EDTA results in the generation of a hollow microcapsule composed of macromolecules surrounded by the polyelectrolyte shell.

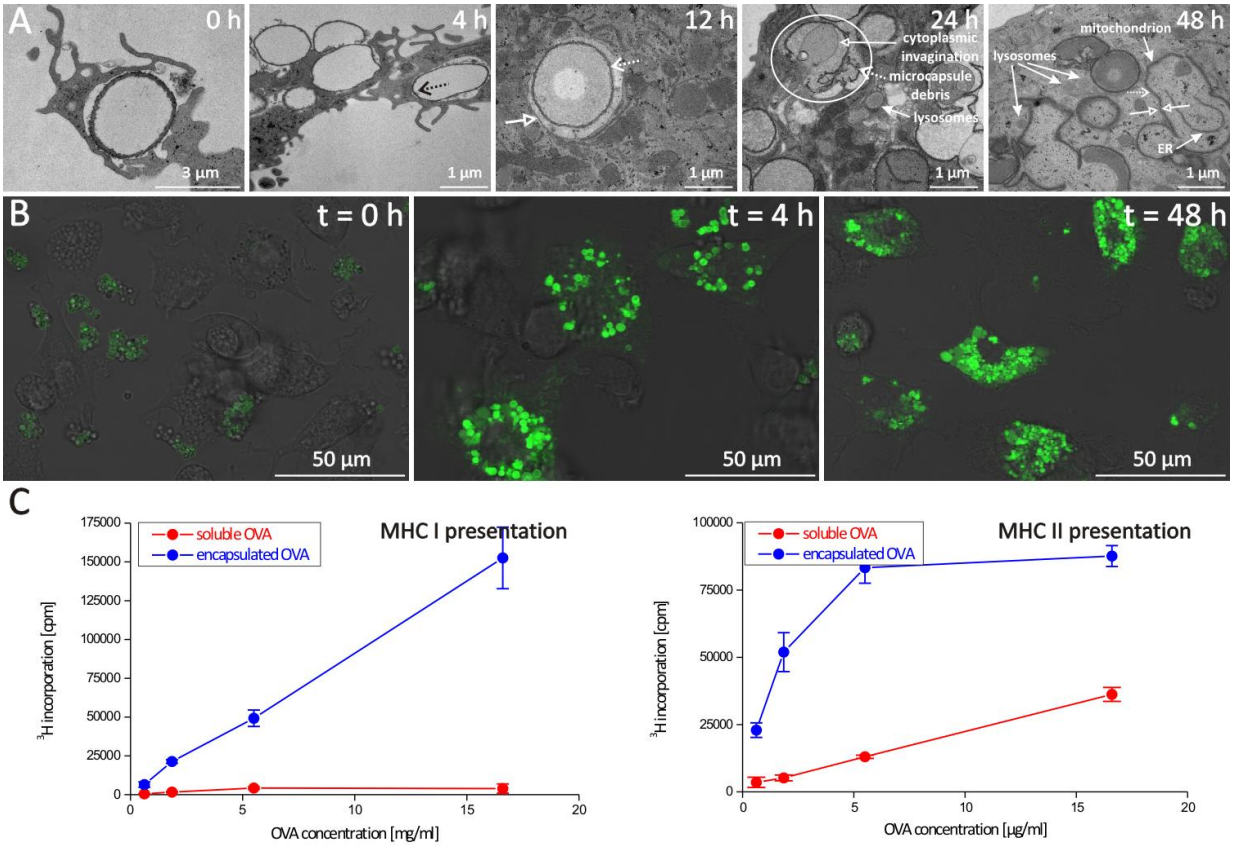

Figure 2. (A) TEM images of BM-DCs that have internalized dextran sulfate/poly-L-arginine microcapsules at the indicated time intervals. Microcapsule shell: dotted arrows; membranes surrounding the microcapsules: open arrows. In the encircled area, microcapsule rupture and cytoplasmic invagination are clearly distinguishable. Lysosomes, endoplasmatic reticulum (ER), and a mitochondrion are indicated by the solid arrows. (B) Processing of dextran sulfate/poly-larginine microcapsule encapsulated OVA was analyzed using DQ-OVA. Confocal microscopy images of BM-DCs incubated with OVA-DQ microcapsules for 0, 4 and 48 h (overlay of green fluorescence and DIC). (DQ-OVA is ovalbumin oversaturated with BODIPY dyes. Upon proteolytic cleavage, quenching is relieved and green fluorescence appears. (C) Antigen presentation by BM-DCs after uptake of soluble and encapsulated OVA. Proliferation of OT-I cells was used as a measure for MHC-I-mediated cross-presentation of OVA (left graph), proliferation of OT-II cells as a measure for MHC-II mediated presentation (right).