Uptake of colloidal polyelectrolyte multilayer capsules by living cells

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INTRODUCTION

Uptake and processing of capsules or particles by cells are important issues with regard to the transport of active agents into the cytoplasm. A recently introduced delivery concept of layer-by-layer assembly of thin planar films (Decher G., 1997) or spherical geometries (Donath E., 1998) of polyelectrolyte multilayer capsules has emerged as a universal carrier system in which cargo and additional functionalities can be introduced as well in the cavities and in the walls of the capsules (De Geest B.G., 2007 ; Zebli B., 2005). Polyelectrolyte multilayer microcapsules functionalized with nanoparticles are ingested spontaneously by cells (Muñoz Javier A., 2006 ; De Geest B.G., 2006). Polyelectrolyte microcapsules are deformed upon the incorporation process due to the mechanical stress caused in the intracellular space. Deformation was dependent on the structure of the capsule walls. Despite deformation, capsules do not lose their cargo even upon compression inside cells. capsules filled with a pH-sensitive dye are taken up by cells and are able to deliver information about the local concentration of protons inside the cells. Thus, the results suggest that internalized capsules are trapped in acidic vesicles rather than free in the cytosol.

MATERIALS AND METHODS

Transmission Electron Microscopy (TEM): Swiss 3T3 Fibroblasts were incubated overnight with capsules1. Cells were embedded in Epon resin and ultrathin slices were prepared. The slices were imaged with 800 - 5000 x magnification in a TEM.

Confocal Microscopy: Human breast adenocarcinoma cells, MCF7, were incubated with capsules 2 and 4. When necessary, the cell membrane was stained with red fluorescent Di-8 Anepps. Images were obtained by series of line scans in two different planes.

Phase Contrast and Fluorescence Microscopy: Human breast carcinoma cells, MDA-MB 435s, were incubated with capsules 3 and 5. Images were taken with a Zeiss Axiotech 200M microscope. Overlay of phase contrast and fluorescence images and the corresponding singles images of different channels were represented.

RESULTS AND DISCUSSION

Are microcapsules incorporated by living cells ? Already previous reports by several groups have indicated that despite their big size (2-5 µm) capsules are indeed incorporated by living cells. This has been demonstrated by confocal microscopy for the different cancer (Muñoz Javier A., 2006 ;

Wang K., 2007) and epithelial cell lines (De Geest B.G., 2006) and bone-marrow derived primary dendritic cells. Though the ingestion of capsules does not cause any acute cytotoxic damage in cell lines (Kirchner C., 2005), animal studies have demonstrated capsules to cause moderate inflammation in tissue.

Capsules deformation upon incorporation by living cells : Polyelectrolyte multilayer capsules are an ideal test system to address the question if deformation of capsules is essential for the uptake process. Polyelectrolyte capsules can be tuned from rigid particles (with intact template core) to relatively soft objects (with removed template core). As well rigid as compressible capsules are incorporated by the mouse embryonic fibroblast cell line 3T3 and the human breast adenocarcinoma cell line MCF 7. The rigid capsules retain their original shape, whereas the flexible capsule are deformed and squeezed upon internalization (Fig. 1).

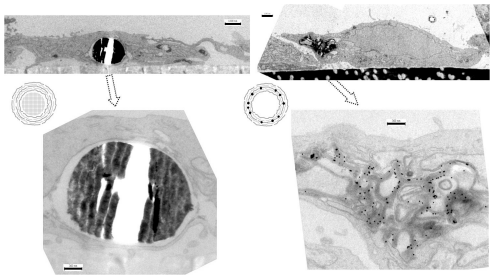

Figure 1: TEM micrographs of internalized Au-nanoparticle modified rigid and soft capsules. 3T3 fibroblasts had been incubated with capsules No. 1 before and after core dissolution on the left and right side, respectively. TEM images of which the scale bars for the overview and detail images represent 1.3 µm and 360 nm, respectively.

Cargo - molecules release upon capsule incorporation : Optical and fluorescence microscopy images with capsules with filled cavity (dextran and Alexa 488-labeled dextran, respectively) show that the cargo is still colocalized with the capsules after their incorporation by cells. These data suggest that though (soft) capsules are deformed and squeezed upon incorporation by cells the cargo in their cavity is not released to the cytosol but is still locally associated with the capsules (Fig. 2).

Intracellular localization of capsules

SNARF-labeled pH-sensitive capsules indicate that internalized capsules are not free in the cytosol of the cells, but are rather confined to locations around the nucleus with acidic pH (Fig 1). Upon internalization, capsules could change their color from red (characteristic for the alkaline cell medium) to green (proper for acidic compartments).

Incorporation of micrometer-sized particles by phagocytotic cells has been described well in literature (Faraasen S., 2003 ; Rejman J., 2004). The mechanical properties of polyelectrolyte capsules can be tuned from rigid particles (with intact template core) to relatively soft objects (with removed template core), as revealed by atomic force microscope measurements (Vinogradova O.I., 2004 ; Dubreuil F., 2003). In contrast to rigid microparticles, (soft) capsules are deformed by cells upon incorporation. Our study and data by others clearly suggest internalization of capsules by cells, even without the presence of specific ligands on the capsule surface.

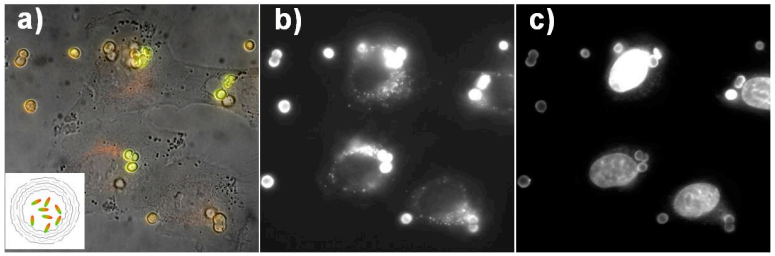

Figure 2: Internalization of SNARF-labeled pH-sensitive soft capsules (no. 3) by living cells. Cells were stained with blue fluorescent DAPI to visualize the nucleus and with red fluorescent FM 4-64 to label the cell membrane. a) Overlay of phase contrast image and fluorescence images of the green and red SNARF-1-channel. Fluorescence image of the b) red FM 4-64 channel and c) blue DAPI channel.

Though some groups have suggested lipid-raft-mediated endocytosis (De Geest B.G., 2006), mainly by excluding other pathways with specific inhibitors, we feel that the uptake mechanism of capsules by cells is yet not fully understood. There are several indications that internalized capsules are not released to the cytosol and are rather trapped in phagosomal structures. This is a slight refinement to our previous statements, where we generally have assumed the capsules to be generally located in endosomal / lysosomal structures (Kreft O., 2007). However, we have to point out that also a different opinion has been raised, in which release of the capsules to the cytosol has been claimed (Reibetanz U., 2006). One problem in this direction to our opinion is that several dyes used to stain the membranes of intracellular structures also directly stain the surface of capsules in cell medium. Due to their size and softness also ingestion pathways different from the ones described in classical textbooks might contribute. Though the pathway of uptake has not been fully elucidated there is significant evidence that for heat-shrunken capsules cargo inside the capsule cavities is not released to the cytosol upon capsule internalization, but is still colocalized with the capsules. This is a very important finding as it suggests that remote release of cargo to the cytosol by remote destruction of the capsule walls (Skirtach A.G., 2006) is not significantly bypassed by uncontrolled release of the cargo during the incorporation process.

Capsule	Process/Method	Polyelectrolyte Layer	Number of layers, n	Cores, ø µm	Core Solvent	Wall attachment	Capsule Cavity
1	Capsule incorporation with TEM	(PDADMAC/PSS)n	2,4,6,8	SiO_2 , 3 µm	0.1 M HF	Au	-
2	Capsule incorporation with confocal microscopy	(PSS/PAH)n	4	Melanin formaldehyde, 4.6 – 6 µm	THF	Alexa 488	-
3	pH-sensitive capsules incorporation with fluorescence microscopy	(PSS/PAH)n	5	$CaCO_3$, 4 – 6 µm	EDTA	-	SNARF-1-dextran (Mw=70 kDa)
4	Capsule localization with confocal microscopy	(PSS/PAH)n	2	$CaCO_3$, 4 – 6 µm	EDTA	TRICT	-
5	Release of cargo with fluorescence microscopy	(PDADMAC/PSS)n	4	SiO_2 , 4.5 µm*	0.1 M HF	Rhodamine	Alexa 488-linked dextran

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CONCLUSION

Our study and data by others clearly suggest internalization of capsules by cells, even without the presence of specific ligands on the capsule surface. In contrast to rigid microparticles (soft) capsules are deformed by cells upon incorporation. Although the present study could not finally address the uptake mechanism, there are several indications that internalized capsules are not released to the cytosol and are rather trapped in acidic structures. Finally, the finding that cargo inside the capsule cavities is not released to the cytosol upon capsule internalization, but is still colocalized with the capsules suggests that remote release of cargo to the cytosol by remote destruction of the capsule walls is not significantly bypassed by uncontrolled release of the cargo during the incorporation process.

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