Polyelectrolyte capsules as multifunctional delivery systems for drug delivery in cells

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INTRODUCTION

Multilayer polyelectrolyte capsules are spherical microcontainers based on layer-by-layer adsorption of oppositely charged polyelectrolyte polymers onto a sacrificial template followed by the decomposition of this template (Sukhorukov, 1998). Polymer capsules have many advantageous properties which make them attractive candidates for medical applications including biosensing and drug delivery. Firstly, they can be synthesized under mild conditions by using numerous different materials. Secondly, their functional properties can be well-defined by embedding different nanoscale building blocks (as colloidal inorganic nanoparticles or biomolecules) within and on top of their wall. Thirdly, they can efficiently host (biological) macromolecules within their cavity for numerous biomedical applications. Finally, they can be composed of biocompatible materials for the delivery of encapsulated materials into cells Rivera (Gil P., 2008). In this manuscript, we describe the light-controlled local release of (macro)molecules from the cavity of internalized capsules into the cytosol of cells without impairing cell viability on the time scale of hours.

MATERIAL AND METHODS

Capsule Synthesis. Polyelectrolyte multilayers were assembled on SiO_2 particles (4.78 µm) by the layer-by-layer deposition technique using solutions of polydiallyl dimethyl ammonium chloride (PDADMAC, 2 mg/mL, 0.5 M NaCl) and polystyrene sufonate (PSS, 2 mg/mL, 0.5 M NaCl), starting from PDADMAC. Following the deposition of the third layer, the particles were resuspended in a solution of gold sulfide/gold nanoparticles (1×10^{15} NPs/mL) (Norman, 2002). The final multilayer composition was PDADMAC/PSS/PDADMAC/AuS_2(PSS/PDADMAC)$_2$/PSS. The sacrificial SiO_2 template was dissolved in hydrofluoric acid (HF, 0.3 M). Solutions of labeled dextran (Mw) 10 kDa (0.5 mg/mL) conjugated to red (Alexa Fluor 594) or blue fluorophores (Cascade Blue) were prepared. Encapsulation of fluorescently labeled dextran was accomplished by first incubating a solution of microcapsules and the desired labeled dextran, and second, by heating the solution to 64 °C for 20 min (Köhler et al., 2006). Exchanging the supernatant with water by carefully sedimenting and resuspending the capsules in water yielded filled capsules, whereby the fluorescently labeled dextran remained inside the wall of polyelectrolyte layers.

Capsule Internalization by Living Cells. A total of 3 × 104 MDA-MB-435s breast cancer cells (ATTC, USA) per square centimeter were seeded on culture dishes (22.1 cm^2, #93060 from TPP) and were incubated with capsules (48×10^4 capsules per cm^2, 16 capsules per cell) for about 24 h. After a washing step to remove free capsules, the culture dish was attached to the x-y-z-stage of the microscope, and cells with incorporated capsules were identified using phase contrast.

Microscopy Setup with Laser for Capsule Exposure. A 100 mWlaser (830nm wavelength) was coupled to an upright microscope (Axitotech, Zeiss, 40× objective) leading to a focused light spot of a few micrometers in diameter in the image plane. By changing the operation voltage of the laser, the resulting power of the focused light spot could be tuned in the range of 0-31 mW. The

microscope was equipped with filters for blue, green, and red fluorescence and with phase contrast. A beam splitter was used to allow for simultaneous excitation of the capsules via the laser and recording the phase contrast and fluorescence images of cells and capsules (Muñoz_Javier, 2009).

Viability Test. In order to probe the cell viability upon opening of capsules, the LIVE/DEAD Viability/Cytotoxicity Assay Kit (from Molecular Probes Invitrogen, catalog number L3224) was applied. This test was either added before or directly after laser-exposure of the capsules (0.1 µL/mL calcein AM 4 mM in anhydrous dimethyl sulfoxide (DMSO) and 0.5 µL/mL ethidium homodimer-1 2 mMin DMSO/H2O 1:4 (v/v)). According to the manufacturer, the kit comprises two probes: calceinAMand ethidium homodimer (EthD-1). CalceinAMis a fluorogenic esterase substrate that is hydrolyzed by esterates to a green-fluorescent product (calcein) and thus, green fluorescence is an indicator of cells that have esterase activity as well as an intact membrane to retain the esterase products. EthD-1 is a high-affinity, red-fluorescent nucleic acid stain that is only able to pass through the compromised membranes of dead cells and thus red fluorescence located in the nucleus is an indicator of dead cells (Muñoz_Javier, 2009).

RESULTS AND DISCUSSION

The ability to mechanically disintegrate metal nanoparticle functionalized capsules depends on the applied power of the light spot. Whereas light spots of 0.7 mW power were typically not sufficient to rupture the wall of capsules, light spots of more than around 2.3 mW power typically lead to deformation and disintegration of the illuminated capsules. For the opening exposure times, less than 1 s were sufficient. Control experiments in which cells were exposed up to 30 min with a light spot of 31mWpower (maximum power output with the setup used) did not show any effect on cells. For experiments with cells, two ranges of laser spot power were used. In the first sets of experiments, the capsules were illuminated with low laser power (2.3 mW) (Figure 1a), the minimum power needed to open the capsules, and with high laser power (31 mW) (Figure 1b), the maximum power output reachable with the laser diode used in these experiments. The phase contrast images reported in Figure 1 show cells that have incorporated capsules (yellow arrows) before and after laser illumination. Red fluorescence images show the cargo release and the nuclear permeation (Ethd-1) in cases where capsules trapped in cells were excited with low laser power and high laser power, respectively. In the case of high power illumination, permeation of the cell membrane leads to loss of fluorescent cargo by diffusion out of the cell. Green fluorescence images indicate decrease of esterase activity in cells where capsules were excited with high and low laser power, respectively.

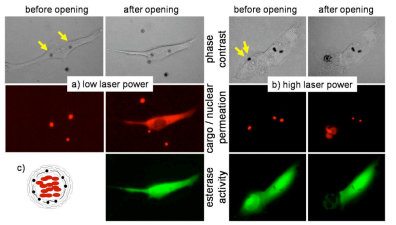

Figure 1. Cargo release and viability/cytotoxicity experiments with capsules filled with red Alexa Fluor 594 dextran as cargo and AuS_2 particles embedded in their walls. (a) Capsules illuminated with low laser power (2.3 mW). (b) Capsules illuminated with high laser power (31 mW). (c) Sketch of the geometry of capsules with Alexa Fluor 594 dextran (red ellipsoids) in their cavity and AuS2 particles (black circles) embedded in their walls.

Typically, after opening of the capsules, a release of the fluorescence labeled dextran to the whole cytosol of the cells was observed. The nuclei of the cells were not stained with the fluorescence labeled dextran. Application of the viability/cytotoxicity kit demonstrated continuous esterase activity of the cells (visible via green fluorescence) after capsule opening for the investigated time periods of up to 2 h after capsule opening. Also no red fluorescence due to ethidium homodimer-1 staining of the nuclei was observed. This corresponds to the data obtained with untreated cells to which the viability/cytotoxicity kit had been applied for up to 2 h. From these data we conclude that the dextran has been released to the cytosol, which consequently must have involved local ruptures in the membranes of the vesicular structures in which the internalized capsules are trapped inside the cells. The viability/cytotoxicity data also suggest that in many, there is no impairment of the viability of the cells within the investigated time of up to 2 h after capsule opening. However, we have to point out that in 30% of our experiments opening of capsules with a laser spot of 2.3mWalso impaired cells, as indicated by decreasing esterase activity and ethidium homodimer-1 staining of the nucleus. The situation changes for higher laser power (31 mW). In this case, after capsule opening, changes in the cell morphology can be frequently observed with phase contrast microscopy. Also the data of the viability/cytotoxicity kit show decreasing esterase activity and ethidium homodimer-1 staining of the nuclei after capsule opening (see the Figure 1). Similar data were found in 100% of the experiments, and they clearly suggest cell death upon light-exposure of the capsules. Whether cell death originates directly from the heat released of the illuminated metal-particles (analogues to hyperthermia) or indirectly from mechanical rupture of the cellular organelles around the capsules cannot be determined from our data.Similar data were obtained for capsules loaded with red and blue fluorescent dextran (see Figure 2). The capsules were illuminated with low laser power (2.3 mW) (Figure 2a), the minimum power needed to open the capsules, and with high laser power (31 mW) (Figure 2b), the maximum power output reachable with the laser diode used in these experiments. The phase contrast images reported in the Figure 2 show cells that have incorporated capsules (yellow arrows) before and after laser illumination. Blue fluorescence images display the fate of capsule's cargo at high and low laser power. Red fluorescence images show nuclear permeation (Ethd-1) in the case where capsules trapped in cells were excited with high laser power, whereas at low laser power, no nuclear permeation can be detected. Green fluorescence images indicate decrease of esterase activity in cells where capsules were excited with high and low laser power respectively.

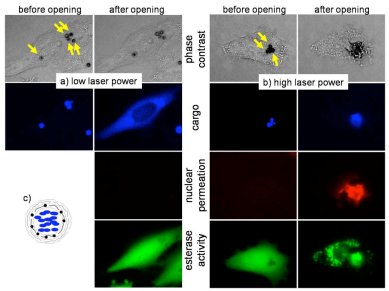

Figure 2. Cargo release and viability/cytotoxicity experiments with capsules filled with blue Cascade Blue dextran as cargo and AuS_2 particles embedded in their walls. (a) Capsules illuminated with low laser power (2.3 mW (b) Capsules illuminated with high laser power (31 mW). (c) Sketch of the geometry of capsules with Cascade Blue dextran (blue ellipsoids) in their cavity and AuS2 particles (black circles) embedded in their walls.

Although in the case of red fluorescence-labeled dextran, there is a spectral overlap between the red fluorescent cargo of the capsules and the red fluorescent ethidium homodimer-1 staining of the nucleus, release of cargo and nuclear staining of dead cells could be distinguished. This is due to the fact that cargo released to the cytosol does not stain the nucleus, whereas ethidium homodimer-1 exclusively stains the nucleus of dead cells. In the case of capsules with blue fluorescent cargo, the problem of spectral overlap was circumvented in first place. These data suggests that there is a window in the applied laser power in which the heat created by illuminated capsules is large enough to locally disintegrate the walls of capsules and surrounding membranes to release the cargo of the capsules to the cytosol, but too low to cause cell death. Though the range of applicable laser power is reproducible, it significantly varies with the batch of capsules used. Inhomogeneities in capsule preparation (presumably due to different metal particle densities in the capsule walls) sometimes lead to destruction of cells after capsule opening with low laser power. However, the majority of cells (70%) in which capsules have been opened with low laser power (2.3mW) are not impaired by the opening procedure. It can be pointed out that thermal energy input in the system is proportional to concentration of nanoparticles, their size and incident laser power (Skirtach et al., 2005). Therefore, the laser intensity range in which cells survive can be tuned by the combination of these parameters.

CONCLUSIONS

Photoactivated release of labeled dextran from the cavity of polyelectrolyte capsules to the cytosol of living cells works with good reproducibility and reliability as demonstrated in this work. Although this is exciting from the technological point of view, arguably dextran is not the most interesting molecule to be released inside cells. Therefore the question arises to which extent the technology platform described here can be transferred for releasing other molecules. However, different cargo molecules might require modified encapsulation strategies, but the encapsulation of cargo in the capsule cavity clearly is not limited to dextran. At any rate, the experiments presented here demonstrate that macromolecules can also be released with flash photolysis using polymer capsules as carrier system. While the system reported here is designed for the opening of individual capsules in single cells, the approach could be generalized for opening a large number of capsules inside tissue. In this case, light-induced heating of Au particles in the shell of the capsules could be replaced by heating of magnetic particles in the shell of the capsules with high frequency fields.

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