Polymer microcapsules as multifunctional carrier systems for sensing and delivery

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INTRODUCTION

In recent years, nano- and microsystems have become very interesting approaches for sensing and delivery in biological and medical applications. The use of nano- or microcontainers such as liposomes or polyelectrolyte microcapsules has reached great interest in this field. Functionalized polymer microcapsules fabricated via Layer-by-Layer (LbL) adsorption of polyelectrolyte materials on spherical templates (del Mercato 2010) are one promising approach to perform as multifunctional carrier system for various applications.

The described capsules serve as cages for the assembly or the separation of compounds that are entrapped in their inner cavities. This could facilitate for instance multiplexed measurements of various analytes (Abbasi 2011). Furthermore, ion-selective fluorophores embedded into these systems are creating an interesting tool for extra- and intracellular ion-sensing applications (del Mercato 2011*). The local ion concentration of various probes can be determined and different capsules can be combined to perform as multiplexed sensor tool (del Mercato 2011).

In addition to the local sensing, the delivery of biological active substances or sensitive dyes to living cells can combine the advantages of delivery and sensor option in one multifunctional tool.
Capsules responsive to external stimuli can act *in vitro* as delivery vehicles (del Mercato 2010). Embedding nanoparticles with energy conversion properties into the polymer shell of such microcapsules enables for light induced opening of the containers and subsequent intracellular release of embedded cargo materials to the cytoplasm (Muñoz-Javier 2008). This technique is also suitable for multiple cargo release such as sequential release of different materials. Furthermore, heat-fragile materials like proteins have been proven to be released from such capsules in vitro without serious loss of functionality (Carregal-Romero 2012).

All these techniques can be easily combined with a targeting approach based on magnetic nanoparticles embedded into polymer microcapsules (Zebli 2005).

MATERIALS AND METHODS

Microcapsules consisting of polyelectrolyte multilayer shells deposed on spherical $CaCO_3$ templates have been fabricated via Layer-by-Layer deposition method. Porous $CaCO_3$ particles are suitable for co-precipitation of cargo moleculeswithin the solid, porous core material. After removal of the calciumcarbonate via chelating agents at mild conditions, resulting polymer microcapsules are enriched with the cargo material. Mainly fluorophores conjugated to dextran molecules of high molecular weight and Proteins have been encapsulated using this method.Alternatively smaller cargo molecules have been successfully encapsulated via post-loading method utilizing irreversible heat-dependent conformation changes of the polyelectrolytes.
Materials for intended intracellular release have been encapsulated as well as functional fluorophores for ion sensing. With these materials ion concentrations of protons, potassium and sodium ions were successfully calculated by ratiometric fluorescence analysis. Multiplexed measurements with all types of sensor capsules in one pot have been performed utilizing fluorescent quantum dots embedded in the polymer shell as bar-code tagging.

Gold nanoparticles possessing energy conversion properties (ability to produce heat upon laser irradiation) were embedded into the polyelectrolyte layers of the shell. Near infrared laser light (830nm) located in the biological window of the electromagnetic spectrum was used for remote controlled opening of so-fabricated capsules. This ultimately led to the release of cargo material to the cytosol of the infiltrated cell culture. Delivery and sensing applications were combined to a multifunctional tool by delivering ion-sensitive probes to the cytosol and determining intracellular pH as well as endosomal proton concentration simultaneously. Furthermore controlled internalization of functional microcapsules via magnetic targeting of capsules modified with magnetic nanoparticles has been proven.

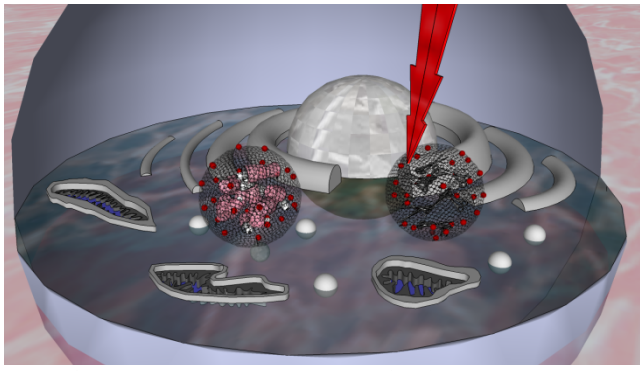

Fig 1: Release strategy of Au nanoparticle modified microcapsules via IR-laser treatment inside living cells

RESULTS AND DISCUSSION

Polyelectrolyte microcapsules have successfully been modified to perform as carrier vehicles for various probes and molecules like fluorophores and proteins. Magnetic targeting was performed utilizing magnetic nanoparticles embedded into the polymer shell of the particles. Different fluorescent probes have been delivered into living cells bytransporting them within microcapsules and releasing them into the cytosol. Infrared laser light has been proven to act as efficient energy source without harming the cell culture or showing significant energy loss in biological tissue. Though light controlled opening of Au nanoparticle modified capsules has been proven to be an efficient release strategy for*in vitro* experiments. Furthermore sequential release of various probes from different capsules in one single cell has been demonstrated. Intracellular release of proteins showed no significant loss in their functionality (fluorescent signal).

Polyelectrolyte capsules have been filled with ion-selective probes to perform as ion-sensors for a variety of possible applications. By embedding fluorescent quantum dots acting as bar-code tagging into the polymer shell such sensor capsules turned out to be a powerful mutliplexing sensor system.

Furthermore, by combining the sensor properties and the release mechanism of polymer capsules a highly sophisticated tool was developed which is capable for analysing intracellular probe concentrations. Releasing further, intracellular active compounds can actively manipulate these simultaneously determined values.

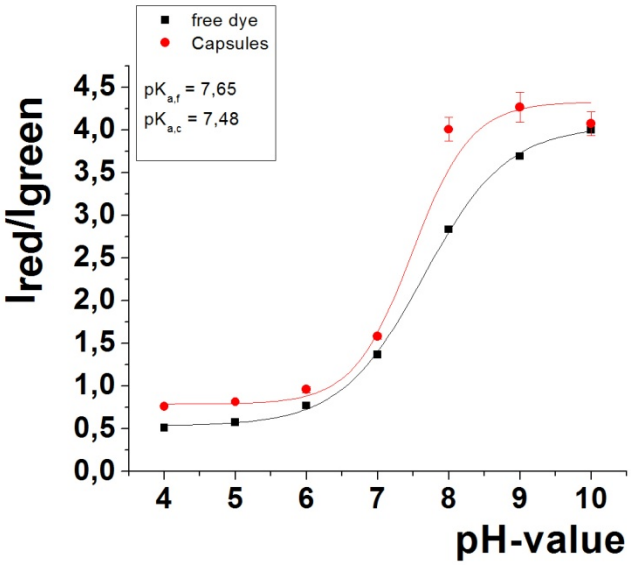

Figure 2: Calibration curve of endosomal and cytosolic proton concentration via ion selective fluorophores analysedinside internalized capsules and after triggered release to the cytosol.

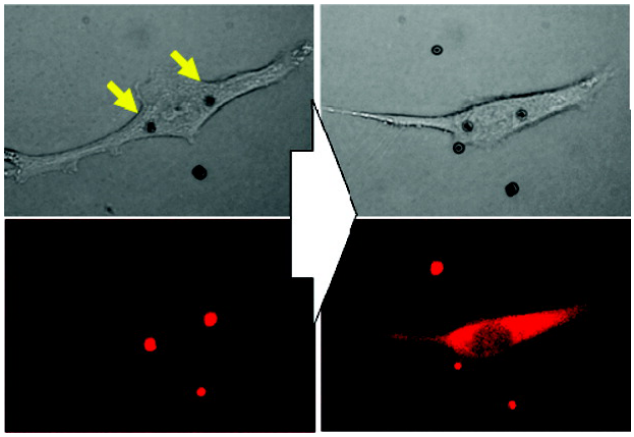

Figure 3 :Release of fluorescently labelled dextran from gold nanoparticle modified polymer microcapsules via laser induced heating.

CONCLUSIONS

Experimental data proof that the presented polymer microcapsules act as very versatile tool for *in vitro* sensing and drug delivery.

Recent investigations indicate for new and exciting possibilities concerning intracellular release of reactive compounds as well as biologically active substances. Further improvements of targeted delivery as well as intracellular reactions triggered by light controlled release of material in living cells are currently in progress.

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