Communication

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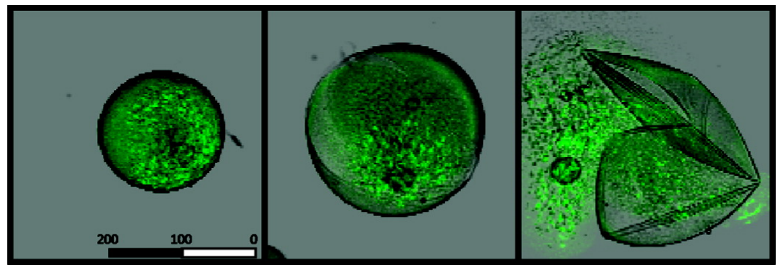

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Microcapsules Ejecting Nanosized Species into the Environment

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Recently, we introduced self-exploding microcapsules, which consist of a degradable dextran-hydroxyethyl methacrylate (dex-HEMA copolymerized with dimethtyl aminoethyl methacrylate (DMAEMA); Figure 1A) microgel core loaded with a macromolecular drug surrounded by a semipermeable membrane (see Figure 1B for a schematic representation).[1] Dex-HEMA hydrogels are degradable by hydrolysis of the carbonate esters which connect the polymerized methacrylate groups with the dextran backbone.[2] When the microgel core degrades, the swelling pressure increases. At a certain moment the surrounding membrane ruptures and the encapsulated material becomes suddenly released from the exploding microcapsule. The self-exploding microcapsules consisted of a microgel core with a mean diameter of 10 μm and a surrounding polyelectrolyte multilayer membrane applied by the well-known layer-by-layer (LbL) technique,[3,4] which is based on the sequential adsorption of charged species onto an oppositely charged surface using electrostatics as the main driving force. The initial aim was to explore the self-exploding microcapsules as a "single shot" vaccine delivery system; we reasoned that a single (simultaneous) injection of microcapsules exploding at different times after injection (as determined by the cross-link density of the microgel core) would release the antigens, encapsulated in the microgels, in multiple pulses.[5]

It can be expected that self-exploding microcapsules may find advanced applications in other fields as well. For example, the sudden release of nanoscopic material after being hosted for a while in a microcapsule at a certain location in a biological tissue (either in the body or growing in vitro) could be attractive.[6] In such applications it could also make sense that the released species become ejected with a high momentum into the biological environment.[7] Molecules or nanoparticles released from microscopic delivery systems reported today have to permeate into surrounding biological tissues by diffusion or convection. As the microcapsules in our study are exploding at a certain time we reasoned that they may give a strong propagating boost to the species they deliver. This would be especially attractive for the release of nanometer-to-micron sized (drug containing) species which show very slow (or even no) Brownian diffusion in biological tissues and thus have difficulties in spreading through tissues.

To develop microcapsules that strongly eject nanoparticles into the environment we aimed to introduce two new features into the self-exploding microcapsule concept reported before. First, we aimed to apply a rigid covalently cross-linked LbL membrane around the microgel core, the reason being that a rigid membrane will not (largely) stretch upon osmotic pressure buildup during the degradation of the microgel. This should lead to a larger pressure difference over the membrane, than in the case where a flexible membrane would be used which would freely stretch during the degradation of the microgel. Therefore, we expect that the encapsulated nanomaterials will experience a strong propagating force, becoming ejected into the environment, at the moment microgel containing capsules surrounded by a rigid membrane explode. Moreover, a covalently cross-linked membrane may prevent leakage of the degradation products of the microgel, which may also contribute to a higher osmotic pressure in the core of the capsules. Note that noncovalently cross-linked LbL coatings are rather permeable and stretchable.[8] Second, to further increase the ejection force we aimed to develop larger dex-HEMA microgels, up to 150 μm in mean diameter.

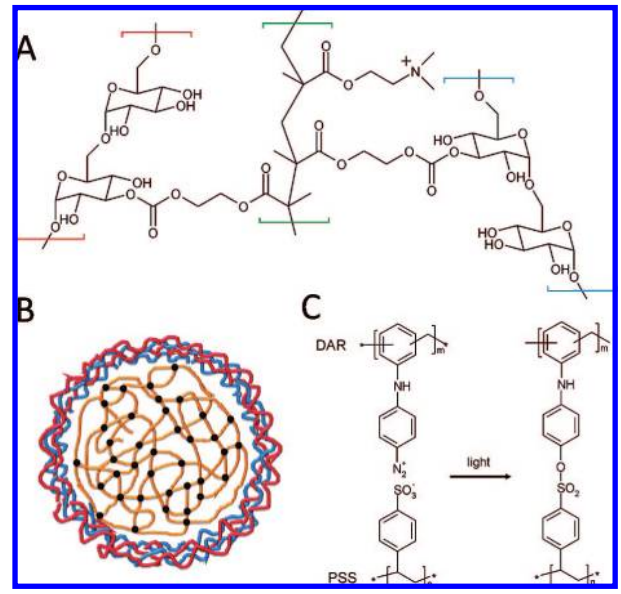

Figure 1. (A) Molecular structure of a dex-HEMA network with DMAEMA groups to introduce cationic charges. (B) Schematic representation of a self-exploding microcapsule with a microgel core, consisting of dextran chains (orange curves) connected by cross-links (black spots), and a membrane consisting of alternating polyelectrolytes (blue and red curves). (C) Molecular structure of the cross-linked PSS/DAR membrane.

Dex-HEMA microgels were fabricated by emulsifying an aqueous dex-HEMA phase into an aqueous poly(ethylene glycol) (PEG) phase.[9] As both phases do not mix a water-in-water emulsion was obtained. Subsequently, radical polymerization of the dex-HEMA's pending methacrylate moieties was initiated, and solid microgels were obtained. To obtain microgels with a cationic surface charge, DMAEMA[9] was added during the emulsification step, resulting in microgels with a ζ-potential of +30 mV. When the dex-HEMA microgels were fabricated by rapid vortexing of the dex-HEMA and PEG phases followed by initiation of radical polymerization, microgels with an average diameter of 15 μm were obtained. It was tested in advance that 15 μm sized dex-HEMA microgels

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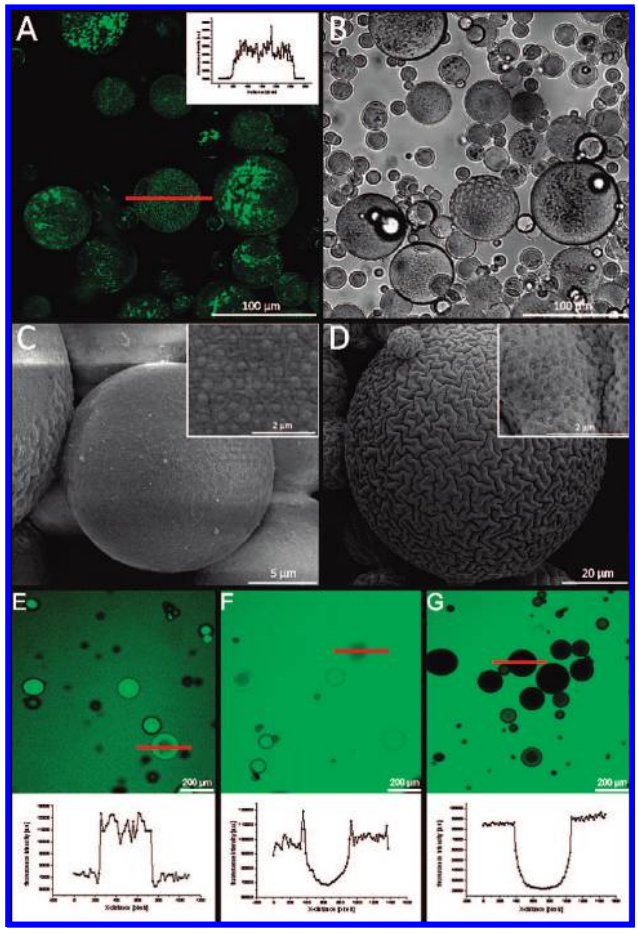

Figure 2. Confocal microscopy image (green channel (A) and transmission channel (B)) of dex-HEMA microgels containing 200 nm carboxylated green fluorescent latex nanoparticles. SEM images of dex-HEMA microgels containing 200 nm carboxylated green fluorescent latex nanoparticles (FITC-NPs). (C) Before LbL coating. (D) After LbL coating. Confocal microscopy images and fluorescence intensity profiles of (E) bare dex-HEMA microgels, (F) dex-HEMA microgels coated with $(PSS/PAH)_4$, and (G) dex-HEMA microgels coated with $(PSS/DAR)_2$ incubated in a 1 mg/mL solution of FITC-dex.

coated with cross-linked LbL layers were not able to explode, probably due to insufficient osmotic pressure upon degradation of the microgel core (data not shown). Therefore, since we were interested in evaluating the effect of the microgel size on the properties of the microcapsules, obtained after LbL coating of the microgels, we modified the emulsification process to vary the microgels' diameter. A pipet tip containing the dex-HEMA solution was immersed into a PEG solution, and the dex-HEMA solution was slowly introduced while the PEG solution was being gently stirred by a magnetic stirring bar followed by the initiation of radical polymerization by addition of KPS/TEMED. By varying the stirring speed, one was able to alter the size distribution of the obtained microgels. In this paper, microgels with a mean average diameter of 150 μm were used (size distribution in the Supporting Information (SI)).

Previous studies showed that dextran based microgels could easily be loaded with macromolecules such as proteins and FITC-dextran.[9] Here we used 200 nm carboxylated green fluorescent latex nanoparticles (FITC-NPs) as species to be released from the self-exploding microcapsules. Therefore, we added the FITC-NPs to the dex-HEMA phase before the emulsification step. The confocal microscopy images of the microcapsules in Figure 2A,B show a punctuated pattern of green fluorescence across the microgels. From these images it can be concluded that FITC-NPs were successfully encapsulated in the microgels. Figure 2C shows the corresponding scanning electron microscopy (SEM) image. The surface of the FITC-NP containing microgels is relatively rough, and in the high magnification image (inset in Figure 2C) individual FITC-NPs can be observed priming through the microgel's surface.

The dex-HEMA microgels were subsequently coated with four bilayers of poly(styrene sulfonate)/diazoresin $(PSS/DAR)_2$. The molecular structures of both polyelectrolytes are shown in Figure 1C. Upon light irradiation the cationic diazo groups of DAR form a covalent bond with the anionic sulfonate groups of PSS resulting in a cross-linked multilayer structure.[10] Figure 2D is an SEM image of $(PSS/DAR)_2$ coated dex-HEMA microgels, further denoted as "microcapsules". Compared to uncoated microgels, the microcapsules show a rough, "brain-like", structured surface which is highly likely due to the differential drying of the microgels and the polyelectrolyte coating prior to imaging, inducing numerous folds in the coating.[1,11] The inset in Figure 2D reveals tiny spots in the $(PSS/DAR)_2$ coating due to the underlying nanoparticles.

Subsequently we studied the permeability of the $(PSS/DAR)_2$ coating: the coating should be permeable to water but impermeable to the degradation products of the microgels, (being 20 kDa dextran chains)[1] to allow (a) buildup of the osmotic pressure upon degradation of the gel and (b) explosion of the capsules at a sufficient value of the osmotic pressure. The permeability of the microcapsules was investigated by incubating them in a solution of 20 kDa green fluorescent dextran (FITC-dex). In control experiments we investigated the permeability of both uncoated microgels and microgels coated with four bilayers of poly(styrene sulfonate)/poly(allylamine hydrochloride), further denoted as $(PSS/PAH)_4$. Figure 2E clearly shows that the (uncoated) microgels are permeable to the FITC-dex. $(PSS/PAH)_4$ coated microgels (Figure 2F) became fluorescent green indicating that the $(PSS/PAH)_4$ membrane is permeable for the FITC-dex. In contrast, when observing Figure 2G, the $(PSS/DAR)_2$ coated microgels can completely exclude the FITC-dex as the microcapsules' interior remains dark indicating that the $(PSS/DAR)_2$ coating renders the microcapsules nearly impermeable to FITC-dex. These findings are in accordance with those of the McShane group who also observed strongly reduced permeability of LbL capsules containing DAR in their membrane.[10]

Next we evaluated whether the $(PSS/DAR)_2$ microcapsules explode upon degradation of the microgel core. A drop of microcapsule suspension was placed under the confocal microscope, and a drop of sodium hydroxide solution (1 M) was added. While under physiological conditions the degradation of the microgels takes days to weeks, depending on the cross-link density of the microgels; the degradation in strong alkaline conditions proceeds within seconds.[2] Figure 3A shows the behavior of $(PSS/DAR)_2$ microcapsules containing FITC-NPs. Upon addition of the sodium hydroxide solution the microcapsules start to swell at a certain moment the LbL membrane cracks. This crack then further propagates leading to explosion of the microcapsule and the release of the encapsulated FITC-NPs. In the introduction we aimed to apply a rigid LbL coating which would swell less compared to a traditional noncross-linked LbL coating. When calculating the swelling ration of the capsules upon degradation of the microgel core a swelling of 28% $\pm$ 2 (n = 3) is observed while the same dex-HEMA microgels coated with a "traditional" poly(styrene sulfonate)/ poly(diallyldimethyl ammoniumchloride) ($(PSS/PDADMAC)_4$) coating exhibited a swelling of 44% $\pm$ 6 (n = 3) (data not shown). Note that PDADMAC was chosen for being a strong polycation which does not lose its charge at high pH (i.e., upon

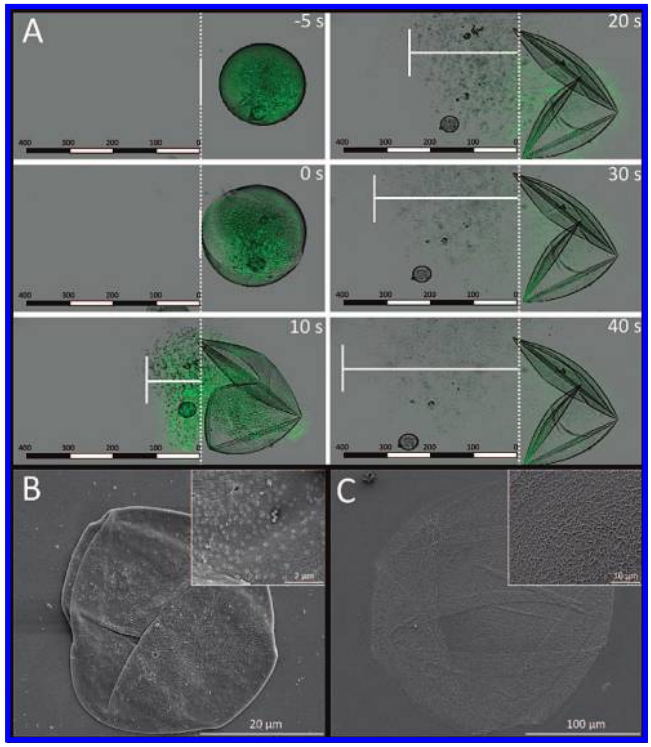

Figure 3. (A) Confocal microscopy snapshots taken at regular time intervals (overlay of green fluorescence channel and transmission channel) of $(PSS/DAR)_2$ coated microgels containing FITC-NP during the degradation of the microgel core triggered by the addition of sodium hydroxide. The microcapsule explodes 10 s after addition of sodium hydroxide, and subsequently the edge of the propagating front of released nanoparticles is marked by the vertical white line. The unit of the scale bar is μm. SEM images of exploded capsules in the case of (B) $(PSS/DAR)_2$ and (C) $(PSS/PDADMAC)_4$ coating. The insets show the surface of the microgels at high magnification.

addition of NaOH) contrary to PAH which is a weak polycation. In the SI a movie is available showing the explosion of the microcapsule. After explosion the $(PSS/DAR)_2$ microcapsules look markedly different from the microcapsules based on lipids, PSS/PAH,[1] or biopolymers (see SI) which we described previously. The open, folded, empty bag remaining after the explosion (in Figure 3A) suggests that the $(PSS/DAR)_2$ membrane is much more rigid. This is also observed by SEM, as shown in Figure 3B: collapsed empty structures can be observed. While the thickness of a "traditional" LbL membrane based on PSS/PDADMAC (Figure 3C) or other polymers is tens of nanometers, the thickness of the $(PSS/DAR)_2$ membrane is hundreds of nanometers.

Remarkably, Figure 3A and the movie in the SI reveal that the nanoparticles are ejected at high speed into the aqueous environment upon explosion of the microcapsule. As can be seen from the time-lapse snapshots in Figure 3A, the NPs travel ~400 μm in 40 s. The Stokes−Einstein equation (eq 1) allows us to calculate the diffusion coefficient (D) of 200 nm sized nanoparticles in water at 25 °C to be 2.5 $\mu m^2/s$.

$$D = \frac{k_B}{6 \cdot \pi \cdot \eta \cdot r} \quad (1)$$

The time required for a 200 nm nanoparticle to overcome a distance of 400 μm in water by simple (one-dimensional) diffusion can be calculated from eq 2 and equals ~32 000 s.

$$D = \frac{\bar{x}^2}{2t} \quad (2)$$

By ejection from the microcapsules, the nanoparticles travel in water almost 800-fold faster than by Brownian motion. Importantly, to our knowledge ejection of species from exploding microcapsules has not been reported before: while drugs/nanoparticles released from all microscopic release devices reported today have to slowly spread into the environment by common Brownian diffusion, species released from exploding microcapsules are propelled into the environment which may allow them to travel relatively large distances in a short time. This feature could be especially attractive in those situations in which the released species have to cross a viscous/low permeable (biological) medium like e.g. mucus covering epithelia, vitreous in the eye, tissue engineered scaffolds, and tissues growing in microfluidic channels.[12]

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Supporting Information Available: Experimental procedures and a movie corresponding to Figure 3A. This material is available free of charge via the Internet at http://pubs.acs.org.

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