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Polymer Nanocontainers

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Abstract: Amphiphilic block copolymers and polyelectrolytes are used to prepare stimuli-sensitive nanocontainers that can be regarded as model systems for host–guest encapsulation and controlled release. Interestingly it is possible to incorporate functional membrane proteins into the walls of these artificial polymer containers. This method can be used to control the exchange of substrates and products of encapsulated enzymes with the external solution, to control virus-assisted loading of the containers with DNA, to apply them as confined reaction vessels for biomimetic mineralization, as nanometer-sized batteries or as molecular motor-driven actuators. This is documented by some representative examples.

Keywords: Amphiphilic block copolymers · Membrane proteins · Polyelectrolytes · Polymer nanocontainers

Introduction

Materials with well-defined structures in the submicrometer region attract increasing interest. The main idea in this context is to tailor composition and function of materials with precise control over size and morphology at the nanometer level, which may lead to new properties for well-known standard materials.

Hollow nanoparticles are particularly interesting for applications as confined reaction vessels, drug carriers or protective shells for enzymes or catalysts [1]. Similar and very effective nanometer-sized containers, *viz*. micelles or vesicles, are already used by nature in biological systems. However, their limited mechanical stability prevents many possible applications (*e.g.* in drug delivery) [2][3]. Recently several promising routes to polymer nanocontainers of higher stability have been developed.

Such nanocontainers have successfully been prepared by polymerization of and in lipid and block copolymer vesicles [4–8] or by multi-step branching reactions leading to dendrimers [9.] Surface-crosslinked hollow polymeric structures can also be obtained by crosslinking the shell of micellar diblock- or triblock copolymer systems and degradation of their core [10–12]. Another possibility for generating polymer hollow spheres is to form a polymer shell around a preformed template particle, which can subsequently be removed. This can be realized by layer-by-layer deposition of opposite-charge polyelectrolytes [13] or by applying interfacial [14], emulsion- [15], suspension- [16] or miniemulsion- [17] polymerization techniques.

In the following we will summarize our own recent efforts to provide routes to stimuli-responsive polymeric nanocontainers based on amphiphilic block copolymers and polyelectrolytes.

Vesicles from Amphiphilic Block Copolymers

Amphiphilic block copolymers consist of at least two parts with different solubilities causing their self-assembly into superstructures in the sub-micrometer range with cores consisting of their insoluble parts surrounded by a corona of their soluble parts [18–21]. This self-organization of block copolymers is based on the same underlying principles as for typical low molecular weight amphiphiles, like surfactants or lipids in water. Block copolymers consisting of hydrophilic and hydrophobic blocks behave in water like conventional surfactants: similar to the latter they self-assemble in water into micelles of various shapes and at higher concentrations into lyotropic liquid crystalline phases. Their aggregation is controlled by hydrophobic interactions and their lyotropic phase behavior by packing constraints of hard sphere objects [22–24].

For a given composition of such block copolymers [4][25–28] (*e.g.* for poly(2-methyloxazoline)-poly(dimethylsiloxane)-poly (2-methyloxazoline, abbreviated: PMOXA-PDMS-PMOXA) triblock copolymers with $M_{n,\,PMOXA} = 1800\ gmol^{-1}$, $M_{n,\,PDMS} = 5400\ gmol^{-1}$) the phase behavior in water is similar to that of typical bilayer forming lipids like lecithin [2][29]. For example, for this triblock copolymer the basic morphological unit are lamellae with a hydrophobic PDMS core and a hydrated PMOXA corona over the whole composition range. Similar to conventional lipids such polymers may form vesicular structures in dilute aqueous solution, which consist of spherically closed block copolymer membranes. Depending on the applied preparation method, the amphiphilic block copolymers could be converted into vesicles with diameters in the range of 50 nm up to about 100 µm.

Recently micromanipulation experiments have shown that block copolymer vesicles are, for example, almost an order of magnitude tougher and sustain far greater areal strain before rupture in comparison to conventional lipid bilayers. Additionally, the polymer membrane has been shown to have a tenfold lower permeability to water than their common lipid analogues [28].

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Similar to conventional lipid vesicles, small unilamellar block copolymer vesicles with sizes in the sub micrometer range can be prepared, for example, by injection and extrusion methods [25][30][31]. Interestingly the average size and size distribution of the resulting vesicles depend not only on the details of the preparation procedure but also on the polydispersity of the block copolymer molecules. In fact there is experimental evidence that in block copolymer vesicles the polymer molecules with shorter hydrophilic block lengths segregate to the inside of the vesicles and long hydrophilic chains to the outside. This segregation increases repulsion between hydrophilic blocks on the outside of the vesicles relative to that on the inside and provides thermodynamic stabilization of the curvature [32]. As a result the vesicles adapt a certain equilibrium size that depends mainly on the molecular weight distribution of the polymers. Moreover, such segregation leads to intrinsically asymmetric membranes with chemically different inside and outside walls of the vesicles, which may have useful applications (*e.g.* for reconstitution of membrane proteins).

Nano- and Microcapsules from Amphiphilic Block Copolymers

The formation of vesicular aggregates from block copolymers is generally a result of non-covalent interactions and, hence, is reversible (even though block copolymer aggregates may be significantly more stable than those formed from low molecular weight amphiphiles). This is, for example, directly reflected in the occurrence of a critical aggregation concentration (cac) below which the vesicles begin to disintegrate and dissolve as individual block copolymer molecules [4]. In case of the reactive PMOXA-PDMS-PMOXA triblock copolymers a crosslinking polymerization of the methacrylate end groups of the underlying polymers can be performed within the vesicles. Then the particles are additionally held together by a covalently crosslinked polymer network structure. As a consequence, the cac vanishes upon polymerization and the resulting nano- and microcapsules possess solid-state properties like shape persistence. Therefore they are able to preserve their hollow sphere morphology even after their isolation from the aqueous solution. It has to be emphasized that during the past few years, extensive efforts have been devoted to the preparation of hollow polymer particles. This is due to their potential for applications in fields like medicine, cosmetics, and pharmacology or as containers for (bio-) chemistry performed on single molecules [33]. In the context of such applications their high stability and shape persistence could be particularly interesting. It would allow, for example, preformed capsules to be loaded with guest molecules in an organic solvent, the isolation of the loaded polymer shells and subsequently the release of the encapsulated material in an aqueous medium.

Nanoreactors from Amphiphilic Block Copolymers

It is obvious that the tendency of the triblock copolymer towards formation of membrane-like superstructures in water closely resembles the behavior of lipid molecules. Hence the block copolymer membranes can be regarded as a mimetic of biological membranes which generally serve as a matrix for membrane proteins (*e.g.* channel proteins) that are responsible for various key functions such as signaling or transmembrane transport. This brought us to the idea to make use of the wealth of naturally occurring channel proteins to control the permeability of such polymer nanocontainers. Recently we could show that these proteins remain fully functional despite the enormous hydrophobic thickness (*i.e.* 10 nm compared to around 3–5 nm for biological membranes!) and stability of the block copolymer membranes and that even after subsequent polymerization of the block copolymer matrix [29][34].

Incorporation of membrane proteins into the shell of (polymerized) triblock copolymer vesicles allows specific molecules to be harvested or separated and also released on demand [35]. The shell can protect encapsulated enzymes against a hostile environment like proteolysis by proteases or self-denaturation and the channels in the shell can be used for 'pre-filtering' the substrates to enhance the sensitivity of the enzyme.

To demonstrate this we incorporated the porin OmpF into the membranes of triblock copolymer vesicles to control the permeability of their shells [34][35]. It is known that molecules with a molecular weight above 400 $gmol^{-1}$ are sterically excluded from these channels. As a representative example we encapsulated the enzyme β-lactamase (MW: 50000 $gmol^{-1}$) in the aqueous core domain of the nanocontainers (see Fig. 1 for a schematic representation). β-lactamase hydrolyzes β-lactam antibiotics like ampicillin (MW: 349 $gmol^{-1}$). In contrast to ampicillin, the product of the hydrolysis, the ampicillinoic acid, can reduce iodine to iodide. Therefore, the full activity of the encapsulated enzyme could readily be proved by iodometry, *i.e. via* the decolorization of a starch-iodine complex. It has to be emphasized that a subsequent polymerization of the nanoreactors did not change their activity within experimental error [35].

Furthermore, the protein OmpF has the interesting property of closing above a critical transmembrane potential. Recently we demonstrated that this gating transition of the protein can be used to switch on or off the nanoreactors *via* external stimuli (see Fig. 1), thus allowing a local and temporal control on the uptake and the release of substrate [35].

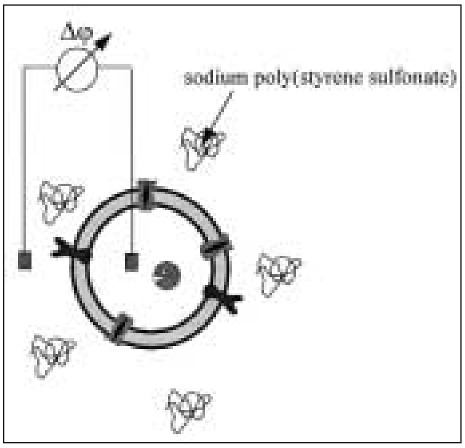

Fig. 1. Schematic view of a nanoreactor with encapsulated enzyme and the Donnan potential induced by polyelectrolyte (Na-PSS: sodium poly(styrene sulfonate) present in the external solution. pH- and voltage-sensitive channel proteins in the shells of the reactors control the exchange of substrates and products.

Another interesting aspect of such polymeric nanocontainers is that the exterior surface is completely covered by the hydrophilic PMOXA blocks of the triblock copolymers which are known to prevent an unspecific protein adsorption (see *e.g.* [2]). Therefore, we expected, at least for long enough hydrophilic blocks, receptors in the walls of such vesicles to be hidden below a hydrophilic polymer layer so that larger ligands would not have access to them. This could be particularly interesting for the use of such nanocontainers as intravasal drug delivery devices. We chose the bacterial receptor protein LamB as a model system for our investigations [36]. This protein forms trimeric channels in the outer cell wall of gram-negative bacteria that allow a specific transport of maltose and maltodextrins. Simultaneously it serves as a receptor for bacteriophage λ. Since during infection the bacteriophages transfer their genome into the host cells it has been proposed that the channels of LamB could be a major intrusion path for the viral DNA. Indeed for

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longer hydrophilic blocks of our polymers the bacteriophages do not find the proteins in the walls of the polymeric containers while they clearly do so for shorter hydrophilic blocks (see Fig. 2). Interestingly the phages transfer during 'infection' their genome into the nanocontainers. Afterwards the DNA-loaded particles could be separated from the phages and purified. These systems could have great potential as vectors for gene therapy, particularly thanks to their small size, low immunogeneity and toxicity and electrically neutral container walls.

Recently we could also show that such polymer nanocontainers are also ideally suited as confined reaction vessels for biomimetic mineralization of inorganic particles of controlled size and morphology. Here we used specific transporter proteins to control the local ion concentration inside the containers during mineralization. As a representative example we encapsulated phosphate ions and then used an ionophore to transport Ca^{2+} ions from the external solution across the polymer membranes into the container interior where calcium phosphate is formed and precipitated [37] (see Fig. 3 for a schematic representation).

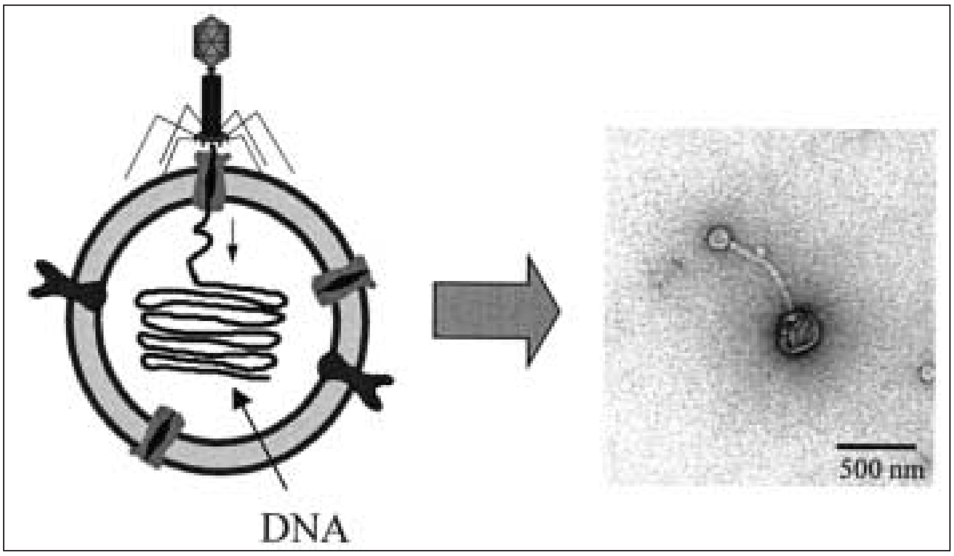

Fig. 2. Schematic representation and TEM-micrograph of a DNA-loaded polymer nanocontainer. A phage binds to a receptor protein and DNA is transferred across the walls of the containers.

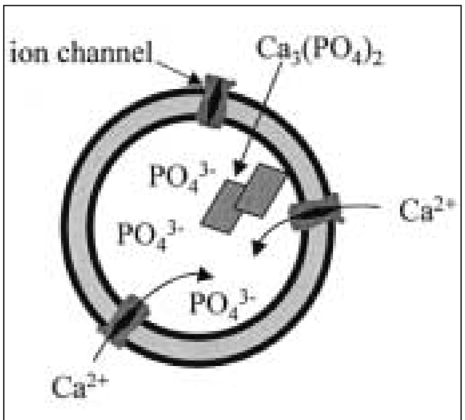

Fig. 3. Schematic representation of an ion-carrier controlled precipitation of calcium phosphate inside a block copolymer nanocontainer.

Polyelectrolyte Nanocontainers as Mimetics of Virion Particles

Our second approach to polymer nanocontainers with a controlled permeability is also inspired by a naturally occurring system. The protein shell of the cowpea chlorotic mottle virus (CCMV) shows a reversible, pH-induced structural transition. Increasing the pH from 5 to 7 leads to a swelling of the virus by approx. 10% [38]. During this swelling gated pores are opened in their shells that allow free molecular exchange between their interior and the bulk medium. This gating has recently been used for a controlled host-guest encapsulation [39]. Although this is clearly a highly fascinating approach, technical applications are not feasible due to the difficulties in handling and producing larger quantities of such virion cages. Therefore we were interested in preparing a simple synthetic mimetic of these virion cages able to undergo a similar structural transition.

The conformation of polyelectrolytes is very sensitive towards changes in ionic strength, pH and other external factors. Hence we selected water-soluble polymer hollow spheres formed by covalently cross-linked polyelectrolyte shells as a model system. The carboxylic groups of poly (acrylic acid) nanocapsules, for example, dissociate increasingly upon raising the pH (see Fig. 4 for a schematic representation). As a result they swell increasingly due to the associated electrostatic repulsion between the identically charged carboxylate anions within their shells. We expected this structural transition to influence considerably the permeability of polyelectrolyte shells similar to the CCMV.

Such nanometer- to micrometer-sized particles can be synthesized, *e.g.* by emulsion polymerization *via* core-shell latexes [40] or by vesicular polymerization [41]. The latter method exploits the fact that the hydrophobic part of lipid bilayer can be selectively swollen by hydrophobic monomers (*e.g.* styrene, alkyl(meth)acrylates). A subsequent crosslinking, free-radical polymerization leads to the formation of a two-dimensional polymer network in the interior of the lipid bilayer. Such polymeric scaffolds increase considerably the mechanical stability of their matrix membranes, without impeding the mobility of the lipids [42].

Due to their crosslinked nature the polymers preserve their hollow sphere morphology after isolation from the matrix vesicles [41][43][44]. While the size and shape of the resulting polymer particles are directly determined by the templating vesicles, the polymer scaffold can be fairly easily modified, using conventional chemical reactions [43][44]. To obtain the desired polyelectrolyte nanocontainers we used as hydrophobic monomers mixtures of *tert.*-butyl acrylate (*t*-BUA) and ethylene glycol dimethacrylate (EGDMA) as the crosslinking agent [44]. After selective saponification of the *t*-butyl ester groups the resulting poly(acrylic acid) particles are dispersible

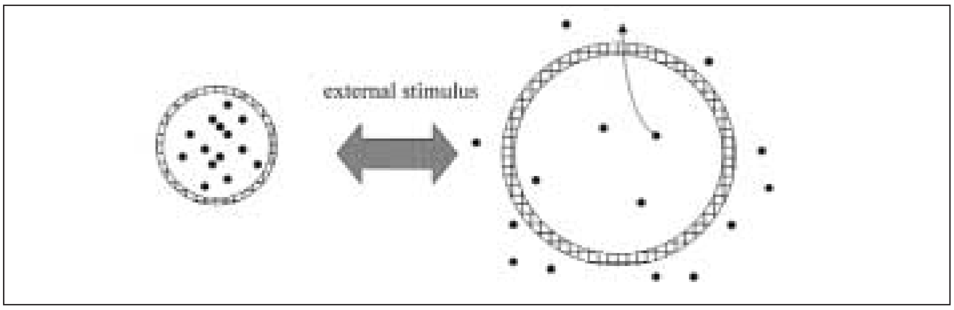

Fig. 4. Schematic representation of stimulus-induced reversible swelling of polyelectrolyte nanocontainers and release of encapsulated material.

in aqueous media. The hollow sphere character of these particles could be proved by cryo-TEM and combined static/dynamic light scattering investigations.

The behavior of a representative sample of poly(acrylic acid) hollow spheres in buffer solutions of varying pH is shown in Fig. 5. As can be seen directly the particle radius increases from about 40 nm at pH <4 to about 190 nm at pH = 8. This corresponds to an increase of the enclosed volume by a factor of about 100! It has to be emphasized that this swelling is completely reversible. The extent of this expansion depends at a given pH additionally on the ionic strength of the buffer, the presence of multivalent ions (*e.g.* Ca^{2+}), the crosslinking density of the polymer network structure within the spherical shells and the presence of hydrophobic comonomers [40][44].

Such pH-sensitive particles can be used to encapsulate water-soluble polymers and dyes, retain the material, *e.g.* at low pH values and release the encapsulated contents again at high pH [40]. Similar to the block copolymer nanoreactors this allows local and temporal control on uptake and release of molecules.

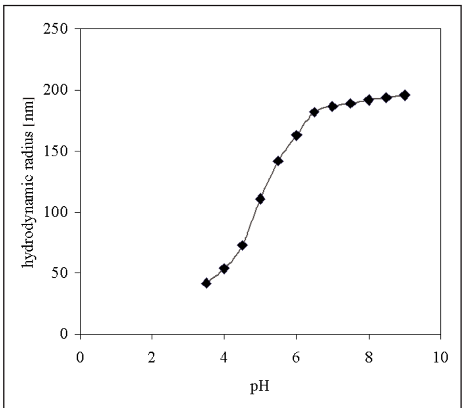

Fig. 5. Hydrodynamic radius of poly(acrylic acid) hollow spheres (3 mol% crosslinker) as a function of pH at constant ionic strength (0.1 M).

Conclusions

It has to be emphasized that both systems should be regarded as representative examples of these types of stimuli-responsive nanocontainers. The possibility to incorporate additional design criteria (e.g. temperature sensitivity, targeting moieties, special surface characteristics) or to combine both strategies is straightforward [45]. In this context it is interesting to note that nature provides many more specific, unspecific or ligand-gated channels (that can additionally be genetically modified) and other membrane proteins, which can be reconstituted in the same way. Preliminary investigations in our lab show that this provides not only a unique tool to control the permeation across the nanocontainer shells but also the potential to use them as molecular motor-driven nanomachines or as nanometer-sized batteries that could be used as power supplies. Moreover, by interconnecting different nanoreactors (containing, for example, otherwise incompatible enzymes) it is possible to prepare nanofactory arrays that are capable to do multistep syntheses. Such systems could be interesting as self-regulating drug delivery devices or as sensors that contain an integrated amplification module for the measured signal.

Generally we believe that the principle of combining the high diversity of polymer chemistry together with the functionality of natural proteins will have many future applications in areas such as drug delivery, sensor technology, energy conversion, diagnostics and catalysis.

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