Hollow Polymer Shells from Biological Templates: Fabrication and Potential Applications

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Abstract: Three-dimensional ultrathin polymer shells have been produced by a combination of step-by-step adsorption of polyelectrolytes on glutaraldehyde-treated human erythrocytes and subsequent solubilization of the cytoplasmatic constituents by means of a deproteinizing agent. The obtained hollow films preserve both the size and shape of the templating cells. This opens a pathway for the fabrication of polymeric capsules within a wide range of size and shape by using various biological templates. They may have exciting potential applications, such as templates for nanocomposites, as containers for a large class of materials, or as cages for chemical reactions. The thickness of the films can be adjusted over a large range: from a few nm up to several tens of nm. The polymer shells are permeable to small molecules and ions but not to macromolecules. An increase in the ionic strength of the solution up to 100 mmol make the capsules permeable for proteins. Permeability and conductivity studies have provided evidence that the adsorption of lipids on polyelectrolyte layers is a means of producing capsules with controlled permeability properties. 6-Carboxyfluorescein and Rhodamin 6G were precipitated within the capsules.

Keywords: cell templating · layered compounds · nanostructures · polyelectrolytes · polymers

Introduction

Supramolecular nanostructures are of considerable interest because of their potential applications in a wide area of technological and biotechnologial processes.[1] A recent technique used for preparing polyelectrolyte structures employs the electrostatic interaction and complex formation between polyanions and polycations.[2] Oppositely charged polyelectrolytes are successively adsorbed onto a charged support. By means of this technique flat macroscopic films have been coated on a variety of materials. A major advantage of this method is that a wide range of polyelectrolyte species can be used as film constituents. In addition, biological macromolecules, surfactants, phospholipids, nanoparticles, inorganic crystals, and multivalent dyes can be incorporated into the polyelectrolyte film.[3] A composite with unique and tailored properties can be thus easily fabricated. X-ray reflectivity, UV-visible and IR spectroscopy, ellipsometry, neutron reflectivity, and quartz crystal microbalance gravimetry have been used to measure film growth and film thickness.[4] Recently, this method of layer-by-layer (LbL) adsorption of polyelectrolytes has been applied to coat-charged colloidal particles.[5] When soluble spherical colloidal particles were used as templates, the solubilization of the core after film formation resulted in the fabrication of spherical closed thin films in a diameter range between 100 nm and 10 μm.[6] Shells of such small dimensions have potential usage as micro- and nanocontainers. Liposomes represent an example of spherical closed thin films, which beside their applications to model biological membranes are, for example, employed as delivery systems in pharmaceutics and cosmetics.[7] Their limited stability and low permeability to polar molecules, however, represent serious limitations for general use.

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Thus it is a challenging task to fabricate organic micro- and nanocapsules in a wide range of size and shape, and with tailored permeability properties. This has been achieved, as shown below, by employing LbL adsorption of polyelectrolytes on biological templates. The encapsulation of cells and other cellular materials may have itself important applications in biotechnology and medicine, because the surrounding polyelectrolyte layer protects the interior from external influences.

In this work we summarize the fabrication of hollow polyelectrolyte shells on erythrocytes.[8] These cells undergo well-defined shape transitions. By templating echinocytes or discocytes shells as microreplicas of those cells can be derived. The shell-wall permeability can be controlled by adsorbing lipids. The permeability and conductance of the shells has been characterized by means of confocal microscopy and electrorotation. The permeability of the capsules for high molecular-weight species can be tuned by changing the ionic strength. Future applications of these novel capsules are outlined by using the fabricated shells as templates for the crystallization of inorganic and organic materials and as reaction vessels for chemistry in small dimensions. The use of biological templates is a step forward towards biomimetical structures, such as "artificial cells". We foresee the development of even more complex molecular assemblies containing functional proteins and lipids.

Results and Discussion

Fabrication and morphology of polyelectrolyte shells from biological templates: A polyelectrolyte layer can be grown on human erythrocytes by alternately adsorbing poly(allylamine hydrochloride) (PAH) and poly(styrenesulfonate) (PSS).[9] Electrophoretic mobility measurements demonstrate the ζ-potential reversal upon adsorption from the third layer onwards, while flow cytometry employing FITC-labeled PAH (FITC = fluorescein isothiocyanate) as the adsorbing species provides evidence of a continuously growing layer.[10] We attribute the absence of the ζ-potential reversal upon adsorption of the first layer to the complex spatial charge distribution at biological surfaces at which the negative surface charge is largely provided by sialic acid residues attached to glycoproteins.[11]

When the desired number of layers were deposited the coated cells were exposed to a deproteinizer treatment.[12] The deproteinizer decomposes the cytoplasmic proteins. At the same time polyelectrolyte molecules in the layer are not destroyed, but altered by the deproteinizing treatment. The low molecular-weight products of the protein decomposition leave the shell interior by diffusing through the shell wall.[13] They can be easily removed by centrifugation or filtration. As a result of this treatment a suspension of polyelectrolyte capsules is obtained. The deproteinizer modifies the chemistry of the layer. Positive charges from the amino groups in PAH are lost, and PSS is partially released. This results in a structure with only negative charges. The layer-by-layer transforms into a network that is created during the oxidation reactions.[14] The oxidation with the deproteinizer results in a pronounced loss of mass from the polyelectrolyte film and in a significantly thinner wall. It is supposed to be more porous than the original polyelectrolyte multilayer film.[15]

The morphology and integrity of the shells were characterized by a number of high-resolution techniques.

In Figure 1 a comparison of two atomic force microscopy (AFM) images is provided, showing polyelectrolyte shells templated on a discocyte (A) and an echinocyte (B).[16] While the more ellipsoidal discocyte templated shells show only few

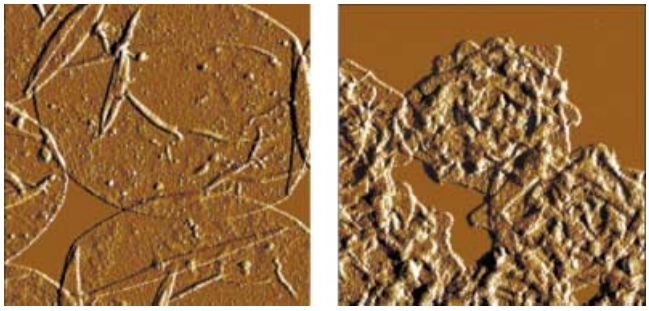

Figure 1. AFM images of polyelectrolyte shells templated on discocytes (left) and echinocytes (right). The shell walls are composed of nine layers. The width of the images is 10 μm. The ellipsoidal discocyte-templated shell shows only a few folds in height less than 50 nm, while the echinocyte shells extend to more than 150 nm in the z direction. The spikes of the echinocytic shell can be clearly distinguished. (The echinocyte is a starlike shape variation of human red blood cells. It can, for example, be induced by a selective expansion of the outer monolayer of the bilayer membrane.)

creases and folds, the templating process on a starlike echinocyte resulted in well-structured capsules that clearly show the spikes of the original template.[17] The produced capsule has two surprising features. First, apart from some deformations it clearly resembles the shape of the original cell, and, second, it shows no traces of cytoplasmic compounds. Even more convincing is the confocal micrograph (Figure 2) providing two cross sections of a capsule formed on

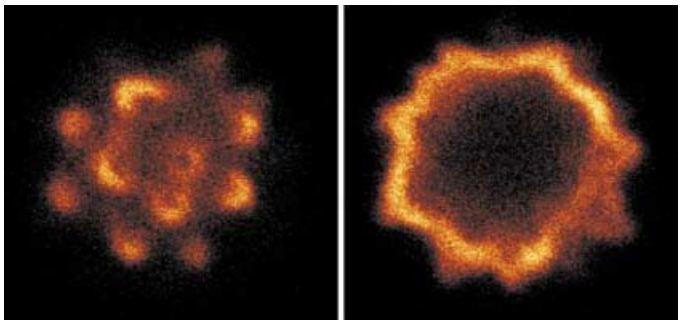

Figure 2. Confocal microscopy scans of a polyelectrolyte shell consisting of 11 layers of PSS/PAH templated on an echinocyte. The outer layer is FITC-labeled PAH. The width of each image is 7 μm. The scans were made in two planes separated by a distance of 1 μm. Left: The scan runs through the upper part of the shell. The polyelectrolyte shell was adsorbed to a glass slide.

an echinocyte. Since this technique avoids drying artefacts it is clear that in aqueous solution the shell is a direct template of the cell. Although close to the limit of optical resolution, it is also clearly distinguishable that the interior of the the spikes

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are hollow. Capsules were also produced from platelets, yeast cells, and bacteria. It can be concluded that the fabrication of polyelectrolyte shells by biological templates opens an exciting pathway towards the production of hollow polymeric shells with defined topology and size.

Polyelectrolyte-shell-wall permeability and conductivity: Among the various possible applications of these novel structures, one of the most promising future aims is to utilize them as micro- or nanocapsules for technological and pharmaceutical application. Regarding this it is most crucial to control the permeability of the shell walls. Experiments with fluorescently labeled polymers have shown that the shell walls are impermeable for water soluble polymers of at least a molecular weight of 4000 and above. In contrast, low molecular-weight compounds, such as ions, small surfactant molecules, and other polar molecules can easily penetrate through the shell walls.[18] To reduce the shell-wall permeability toward small polar molecules, lipids have been adsorbed on top of the polyelectrolyte shell. The adsorption of charged or zwitterionic lipids (for example, dipalmitoyl phosphatidic acid (DPPA) or dipalmitoyl phosphatidyl choline (DPPC)) was either achieved through adsorption of preformed vesicles on the shells or by solvent exchange starting from a lipid–methanol solution.[19] Figure 3 shows a confocal microscopy image of lipid-coated polyelectrolyte shells in which the polar marker 6-carboxyfluorescein (6-CF) has been added to the aqueous solution. It can be seen that the lipid coating prevents the 6-CF from penetrating into the shell interior. Our experiments revealed further that such lipid layers were stable for at least eight weeks and that further polyelectrolyte layers can be added on top of lipid layers.

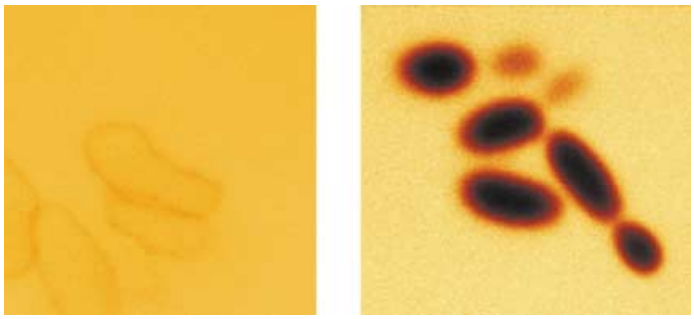

Figure 3. Confocal images of polyelectrolyte shells templated on discocytes. The shell walls were prepared by assembling ten layers of PSS/PAH. The shells were exposed to a solution of 6-CF (100 μM). The fluorescent 6-CF molecules penetrate into the shell interior as seen by the fluorescence intensity within the shells (left), while an additional deposition of DPPA prevented 6-CF from permeation into the shells as can be deduced from the dark appearance of the capsule interior (right). DPPA was deposited onto the capsule walls by means of exchanging methanol for water.[19] The widths of the images are 16 μm (left) and 14 μm (right).

The conductance of control and lipid-coated polyelectrolyte capsules has been measured by means of electrorotation.[20] While the conductance of the bare polyelectrolyte multilayer that consists of ten layers was approximately 1 Sm^{-1}, lipid-coating resulted in conductance values of the composite layer of less than 10^{-4} Sm^{-1}.[21]

The high permeability of the capsule walls to low molecular-weight compounds is useful for the synthesis and encapsulation of macromolecules and inorganic nanoparticles inside polyelectrolyte capsules. For example, a number of polymers were synthesized by means of radical polymerization from water-soluble monomers inside the capsules as well as in the bulk. Removal of the excess bulk polymer yielded a suspension of capsules that contained the polymers at desired concentrations.[22]

The polyelectrolyte capsules may become permeable for high molecular-weight species like polymers or proteins by increasing the ionic strength in solution. In Figure 4 capsules in a salt-free solution of FITC-labeled albumin (Mw 70000) are shown. From the absence of fluorescence inside it can be concluded that the proteins are excluded from the capsule interior. In the presence of 100 mmol NaCl, however, the capsules open after a few minutes for albumin, which is proved from the fluorescence inside the capsules.

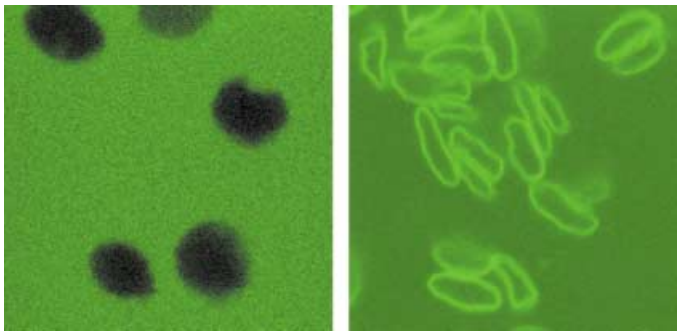

Figure 4. Confocal images of polyelectrolyte capsules templated on discocytes. The shell walls were prepared by assembling five layers of PSS/PAH. The shells were exposed to FITC-labeled albumin solution (1 mg mL^{-1}). Left: The labeled protein does not penetrate in the capsules as seen from the darkness inside the capsule wall. Right: The addition of NaCl in a concentration of 100 mmol made the capsules permeable for the protein, which follows from the appearance of fluorescence in the capsule interior. The width of each image is 25 μm.

The permeability increase becomes noticeable at a salt concentration of approximately 5 mmol and is probably related to changes in the wall structure generated as a result of the deproteinizer treatment.[23]

Controlled precipitation inside capsules: The anisotropic shells were used as templates for controlled precipitation or crystallization of organic and inorganic materials. To illustrate this, polyelectrolyte shells templated on discocytes were incubated in a solution of 6-CF (30 mmol) at pH 7. Then the pH was dropped to a value of 3.5, at which 6-CF becomes largely insoluble. Incubation over 1–12 hours yielded a mixture of empty and shells completely filled with 6-CF. Not much, if any, precipitation was found outside the shells. Figure 5 shows a transmission microscopy image and an SEM image of the shells filled with 6-CF.[24] The filled shells appear completely dark in transmission. The SEM image shows that the precipitated 6-CF together with the surrounding polyelectrolyte shell yielded a structure that is very similar in size and shape to the original template. In a similar manner

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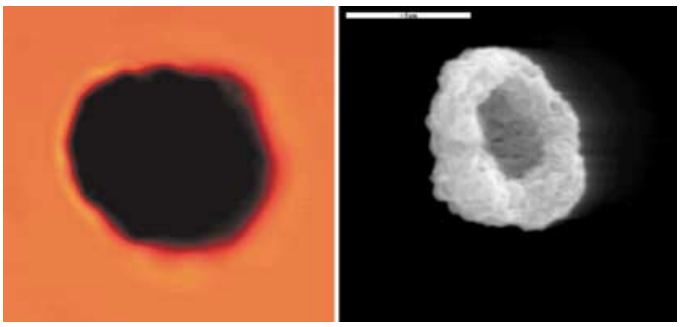

Figure 5. Transmission light microscopy image of a polyelectrolyte shell (ten layers) templated on discocyte (left) and the corresponding SEM image filled with precipitated 6-CF (right). The completely dark image (left) is explained by the strong absorbance of the crystallized 6-CF. The SEM image demonstrates that the 6-CF precipitated in the shell interior assumes the shape of the original template, see Figures 2 and 3. The width of the left-hand image is 8 μm. The bar in the right-hand image is 5 μm

rhodamine 6G and a variety of inorganic salts were selectively precipitated inside the polyelectrolyte capsules. Although not every detail of the selective precipitation process is understood yet, it seems to be important that nucleation starts at the inner surface of the capsule walls. For example, in the case of 6-CF the negative charge of the outer surface inhibited adsorption of the dye to the capsule surface. After the onset of crystallization the bulk solution soon becomes depleted from 6-CF; this further reduces the probability of forming crystals outside the capsules. The crystallization stops when the shell has been completely filled. Ostwald ripening may then take place, producing completely filled shells at the expense of partly filled shells.[25]

Conclusion

The novel technique of the step-wise self-assembly of polyelectrolyte multilayers onto biological templates with subsequent solubilization of the templating core yielded polyelectrolyte shells with controlled size and shape, as they are replicas of the original cells. This result is a significant step toward the aim of the fabrication of well-defined supramolecular structures in nanometer dimensions for multipurpose applications. The oxidation process resulted in a new material with properties different from the assembled polyelectrolyte film. The combination of polyelectrolyte multilayers and lipid layers allowed us to control the permeability of small polar molecules. The deposition of lipids in ordered layers yielded stable systems, which may be used for biotechnological applications. The use of these novel structures for biomimetic precipitation processes is demonstrated by producing organic and inorganic composites that were predetermined in size and shape by the original polymer shell. In this respect, the control of the chemical composition of the polymer layer is an important advantage to meet crystallization conditions for various substances. Recently it has been shown that the shells can be used as carriers for various solvents.[26] The incorporation of biological functions into these structures is expected to come in the near future. This opens up novel ways for the fabrication of interesting materials.

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[8] The cytoplasmic membrane instability upon adsorption of strongly charged polyelectrolyte molecules has been overcome by pre-treating the cells with a cross-linking agent such as glutardialdehyde. The cells were incubated in 2% v/v glutaraldehyde for 60 min prior to the coating process.

[9] Typical adsorption conditions employed 20 mmol polyelectrolyte (PAH, Mw 50000, or PSS, Mw 70000) in NaCl (0.5 M), pH 6, at cell concentrations of 2.5% v/v. The adsorption time was 10 min. A 2.5% v/v cell suspension in NaCl (0.5 M) with either PAH or PSS (0.5 mg mL^{-1}) was incubated for 10 min. The resulting solution was washed with NaCl (3 × 145 mmol) prior to the next adsorption step.

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[12] The coated cells were treated with a 1.2% w/v NaOCl solution for 20 min to remove the cytoplasmic constituents; the cells were then washed with NaCl (3×145 mmol).
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