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Permeation of Macromolecules into Polyelectrolyte Microcapsules

R. Georgieva,[†] S. Moya,[‡,§] M. Hin,[†,||] R. Mitlöhner,[†] E. Donath,[‡,§] H. Kiesewetter,[†] H. Möhwald,[‡] and H. Bäumler*[,†]

Institute of Transfusion Medicine, Medical Faculty Charité, Humboldt University of Berlin, Berlin, Germany, Max Planck Institute of Colloids and Interfaces, Golm/Potsdam, D-14476, Germany, and Leipzig University, Institute of Medical Physics and Biophysics, Leipzig, D-04103, Germany

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Polyelectrolyte microcapsules (PEMCs) have been prepared by coating red blood cells with the polyelectrolytes poly(styrenesulfonate), poly(allylamine hydrochloride), and dextran sulfate applying the layer-by-layer technique with subsequent dissolution of the core. The capsule permeability for human serum albumin (HSA) was studied as a function of the ionic strength and pH by means of confocal microscopy. PEMCs produced with dextran sulfate and poly(allylamine hydrochloride) show a significant increase in permeability for HSA at salt concentrations over 1 mM. For PEMCs prepared with poly(styrenesulfonate) and poly(allylamine hydrochloride) the limiting salt concentration is 5 mM. No pH dependence for permeation was observed. A correlation between the permeation and adsorption of HSA on the PEMC walls was investigated. Finally, a mechanism for the permeability, combining electrostatic interactions, and the presence of pores in the polymer layers is presented confirmed by the considerable increase of permeation of charged molecules in the presence of salt and the permeation of neutral molecules regardless of the ionic strength.

Introduction

Recently, micro- and nanosized polyelectrolyte capsules have been fabricated by applying the layer-by-layer adsorption technique[1] on charged colloidal particles with subsequent decomposition and removal of the core.[2−4] The size of the capsules can be varied from 0.1 to tens of micrometers, and it is defined by the size of the template. The thickness of the capsule wall depends on the number of assembled polyelectrolyte layers, and it can be adjusted in the nanometer range.[5] A variety of colloidal particles, melamine resin latexes (MRLs),[2−4,6,7] biological cells,[4,9,10] and organic[11,12] and inorganic crystals[13,14] have already been used as templates for the capsule preparation.

Melamine resin particles and crystal templates are usually dissolved by means of a pH change. Red blood cells (RBCs) and other biological templates are decomposed by applying an oxidizing sodium hypochlorite solution (deproteinizer).[9] Both the pH change and the oxidation process result in additional changes of the structure and properties of the polyelectrolyte multilayer capsule wall as compared with the original polyelectrolyte film assembled on the particle surface.[15] In the fabrication of the RBC capsules with deproteinizer both the biological template and the assembled polyelectrolyte layers are oxidized. This results in a loss of amino groups in the film as well as in a significant mass reduction.[15] In addition, the oxidation and release of biological material cause a transient osmotic expansion.[16,17] The reversibility of the expansion is explained by the elasticity of the shell wall representing a polymer net held together by bonds formed during oxidation.

The novel capsules may have applications in biology and medicine as microcontainers and microreactors for drugs, enzymes, DNA, and other bioactive substances. Their size and shape and their mechanical and chemical stability mimic that of the cells. Especially, polyelectrolyte microcapsules (PEMCs) fabricated on RBCs are very interesting for this purpose because their elastic properties and their shape largely match the original biological template.[9]

In view of their potential application as containers for bioactive materials with the size of a cell, it is essential to understand the interaction of PEMCs with biomolecules. The understanding and control of the capsule permeability for these molecules are the basic requisite for any future application of the PEMC as drug delivery systems.

It has been already shown that small polar molecules and ions permeate the polyelectrolyte capsule wall.[11,12,18] The permeation of polymers into polyelectrolyte capsules assembled on MF latex particles depends on a variety of parameters of the bulk solution such as ion concentration,[19] pH,[7] and temperature.[20] Because of the basically different composition and architecture of the PEMCs fabricated on biological cells compared with the capsules templated on MF latexes, different permeability properties can be expected.

The aim of this work was to study the permeation of biomacromolecules into preformed PEMCs templated on RBCs in dependence on the salt concentration and pH. Special attention was paid to the relation between adsorption

* To whom correspondence should be sent.
† Humboldt University of Berlin.
‡ Max Planck Institute of Colloids and Interfaces.
§ Leipzig University.
|| Part of the results obtained in this work are based on the thesis of M.H.

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of macromolecules on the surface of the capsules and their subsequent penetration into the capsule interior.

A new polyelectrolyte combination of biocompatible dextran sulfate with poly(allylamine hydrochloride) was used for capsule preparation. The permeability of these new PEMCs was compared with that of PEMCs made of the system poly(styrenesulfonate)/poly(allylamine hydrochloride).

Materials and Methods

Materials. The sources of chemicals were as follows:

Polyelectrolytes: poly(styrenesulfonate, sodium salt) (PSS), M_w 70000, Aldrich; poly(allylamine hydrochloride) (PAH), M_w 70000, Aldrich; dextran sulfate ($DxSO_4$), M_w 500000, Pharmacia Fine Chemicals, Sweden; human serum albumin (HSA), M_w ca. 65000, Sigma.

Labeled substances: fluorescein isothiocyanate labeled human serum albumin (FITC−HSA), fluorescein isothiocyanate labeled dextran (FITC-Dextran), M_w 77000; Sigma.

Other chemicals: glutaraldehyde (Grade I), Sigma-Aldrich; sodium hypochlorite (NaOCl), sodium chloride (NaCl) and phosphate buffer solution (PBS).

The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm.

PEMC Preparation. *Erythrocyte Fixation.* Erythrocytes were obtained from fresh human blood anticoagulated with ethylenediamine tetraacetate (EDTA) by means of centrifugation and subsequently washed twice in buffered NaCl solution (140 mM NaCl, 5.6 mM KCl, 5.8 mM sodium phosphate buffer, pH 7.4). The cells were then stabilized with glutaraldehyde[21] at a final concentration of 2% for 60 min at 20 °C. After fixation the cells were washed at least four times with distilled water and then resuspended in 154 mM NaCl solution.

Polyelectrolyte Film Assembly and Core Dissolution. The stepwise adsorption of oppositely charged polymers was performed using a filtration technique.[4]

The polyelectrolyte assembly was performed with either PSS or $DxSO_4$ as the negatively charged polyelectrolytes followed by PAH as the positive polyelectrolyte. The coating was always started with the negative polymer.

Fixed erythrocytes were suspended in buffer-free 1 mg/mL polyelectrolyte and 0.5 M NaCl with a final cell concentration of 10% (v/v). The pH values were 5 ± 0.1, 5.5 ± 0.1, and 6.0 ± 0.2 for the PAH, PSS, and $DxSO_4$ solutions, respectively. The cells were incubated under slight stirring for 15 min at room temperature. Washing the samples twice in a 100 mM NaCl (pH 6.5−7.0) solution finished each step.

For the preparation of the PEMCs, erythrocytes with 5, 7, or 15 layers $(PSS/PAH)_2PSS$, $(PSS/PAH)_3PSS$, $(PSS/PAH)_7PSS$, or $(DxSO_4/PAH)_2DxSO_4$, $(DxSO_4/PAH)_3DxSO_4$, $(DxSO_4/PAH)_2DxSO_4$, respectively, were suspended in a solution of 140 mM NaCl and 1.2% NaOCl. Within 20 min of incubation at 20 °C the cellular template was dissolved obtaining hollow polyelectrolyte microcapsules.[9]

Afterward the sample was washed three times with a 154 mM NaCl solution and additionally with distilled water until a supernatant conductivity of 0.5 mS/m was reached. Since the capsules are permeable for small ions, rinsing with water does not induce any osmotic response.

Confocal Laser Scanning Microscopy. Confocal images were taken with a confocal laser-scanning system TCLS attached to an inverse microscope from Leica (Wetzlar, Germany), equipped with a 100× oil immersion objective with a numerical aperture of 1.4.

PEMCs were scanned at different heights before classifying them as filled or not filled. Those classified as filled showed fluorescence in the middle plane.

The fluorescence intensities of the scanned capsules were measured by applying the TCLS software.

Scanning Force Microscopy. Scanning force microscopy (SFM) images have been recorded in air at room temperature using a Nanoscope III Multimode SFM (Digital Instrument Inc., Santa Barbara, CA) in contact mode. Microlithographed tips on silicon nitride (Si_3N_4) cantilevers with a force constant of 0.58 N/m (Digital Instrument) have been used. SFM images were processed by using the Nanoscope software. Samples have been prepared by applying a drop of the PEMC suspension onto a freshly cleaved mica substrate. After allowing the PEMCs to settle, the substrate was extensively rinsed in water and then dried under a gentle stream of nitrogen.

Electrophoretic Mobility Measurements. The electrophoretic mobility of the polyelectrolyte-covered erythrocytes as well as of the PEMCs was measured by means of an electrophoresis cell/particle analyzer[22] (Electrophor, Hasotec, Rostock, Germany). The procedure has been described in detail elsewhere.[23]

Results and Discussion

The permeation of macromolecules was followed by confocal microscopy using labeled substances. PEMCs consisting of $(PSS/PAH)_2PSS$ and $(DxSO_4/PAH)_2DxSO_4$ as polyelectrolyte components were investigated and compared. As it was mentioned in the Introduction there is a dramatic change in the chemical composition after digestion of the erythrocyte template. Capsules made of PSS and PAH lose almost all PSS present in the film before the oxidation. The sugar rings of $DxSO_4$ are also easily oxidized. As a result of this mass loss, similar permeation properties for both capsule kinds were expected.

Figure 1 shows confocal micrographs of PEMC obtained by adsorbing five polyelectrolyte layers, $(PSS/PAH)_2PSS$. The capsules are suspended in aqueous solutions of FITC-albumin at various NaCl concentrations of 0, 1, 10, and 50 mM. It can be seen that 10 min after suspension the capsule interior remains dark in the absence of salt. The fluorescence probe, FITC-albumin, is excluded from the capsules up to a salt concentration of 1 mM. At higher NaCl concentrations, as illustrated for 10 and 50 mM, the fluorescence intensity in the capsule interior within 10 min becomes equal to or even apparently higher than the intensity of the probe in the bulk.

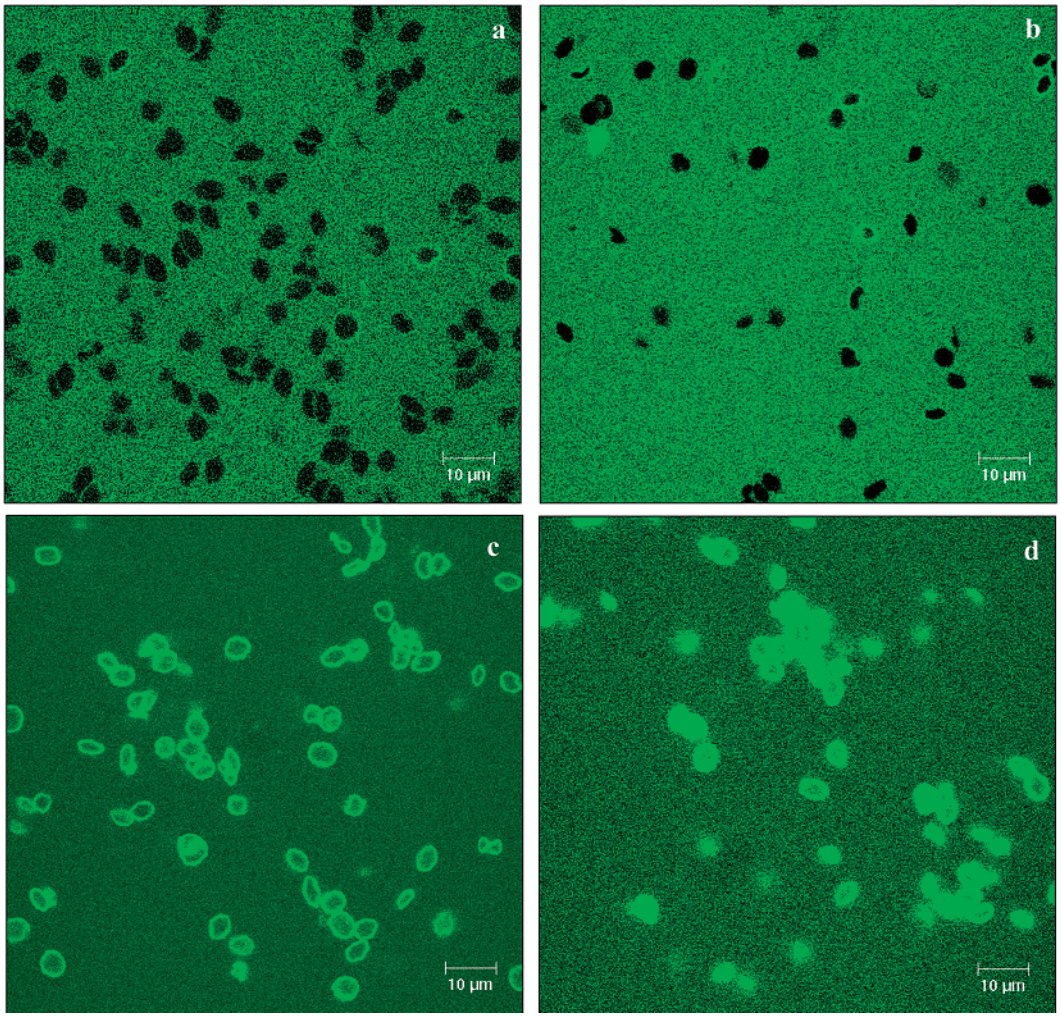

Figure 1. CLSM images of $(PSS/PAH)_2PSS$ capsules in FITC-HSA solutions in (a) distilled water, (b) 10^{-3} M NaCl, (c) 10^{-2} M NaCl, and (d) 5×10^{-2} M NaCl. The concentration of FITC-albumin in the suspension was 1.8 mg/mL. All images were taken 10 min after the capsules were mixed with the FITC-albumin solution.

In parallel to the increasing fluorescence intensity in the capsule interior, one can observe a much stronger fluorescence coming from the capsule wall with increasing NaCl concentration. There was also no principle difference in the behavior of PEMC fabricated with 7, 9, or 15 polyelectrolyte layers.

It is obvious that albumin very easily permeates through the polyelectrolyte wall when the salt concentration is high enough but does not permeate within 10 min into the capsule interior in water and in solutions up to a salt concentration of 1 mM. Only after a long incubation time of the order of hours HSA permeates into the capsule interior. A similar dependence of permeability on salt concentration was found by Ibarz et al.[19] for capsules templated on MF particles. The authors discussed a possible mechanism of opening pores in the polyelectrolyte film due to conformational changes of the polyions in dependence on the salt concentration.

The observation of higher fluorescence intensity coming from the capsule wall is certainly a result of adsorption of albumin molecules onto the wall of the polyelectrolyte capsule in the presence of salt. The apparent correlation of adsorption and permeability increase is intriguing. It was interesting to learn, whether the underlying mechanism for both processes has a common basis or whether our findings represent just a coincidence.

Let us first discuss the adsorption behavior of albumin since this phenomenon has been already studied on macroscopic flat surfaces covered with polyelectrolyte films. Recent investigations of PSS/PAH films on silica have shown that the adsorption of albumin molecules occurs both on positively and on negatively charged surfaces.[24,25] It was concluded that mainly electrostatic interactions determine adsorption. For positively charged surfaces, the thickness of the protein film exceeded several times the largest dimension of the protein molecule. In the case of negatively charged surfaces, a dense protein monolayer at pH 7.4 was found.

The charge of the PEMC is also negative in the pH range from 4.1 to 7.4 as can be seen from electrophoretic measurements. In Table 1 the electrophoretic mobilities of $(PSS/PAH)_3PSS$ and $(DxSO_4/PAH)_3DxSO_4$ capsules at pH 4.1 and 7.4 are compared with the mobilities of the templates (glutaraldehyde stabilized erythrocytes) and the template after layer-by-layer covering with four negative (PSS, respectively $DxSO_4$) and three positive (PAH) layers. It can be seen, that the PEMC mobility is not very different from the mobility of the covered template but is about twice as large as the

Table 1. Electrophoretic Mobilities of Stabilized RBC, RBC Coated with Plyelectrolyte Layers, and Polyelectrolyte Capsules in 300 mOsm PBS (145 mM NaCl, 5.8 mM phosphate buffer) at Two Different pH Values

	electrophoretic mobility 10^{-8} m $^2 \cdot s^{-1} \cdot V^{-1}$				
pH	RBC	RBC with $(PSS/PAH)_3PSS$	PEMC $(PSS/PAH)_3PSS$	RBC with $(DxSO_4/PAH)_3DxSO_4$	PEMC $(DxSO_4/PAH)_3DxSO_4$
4.1	-1.18 ± 0.04	-3.02 ± 0.06	-2.53 ± 0.12	-2.76 ± 0.05	-2.24 ± 0.22
7.4	-1.29 ± 0.05	-2.98 ± 0.06	-2.82 ± 0.30	-2.80 ± 0.08	-2.69 ± 0.22

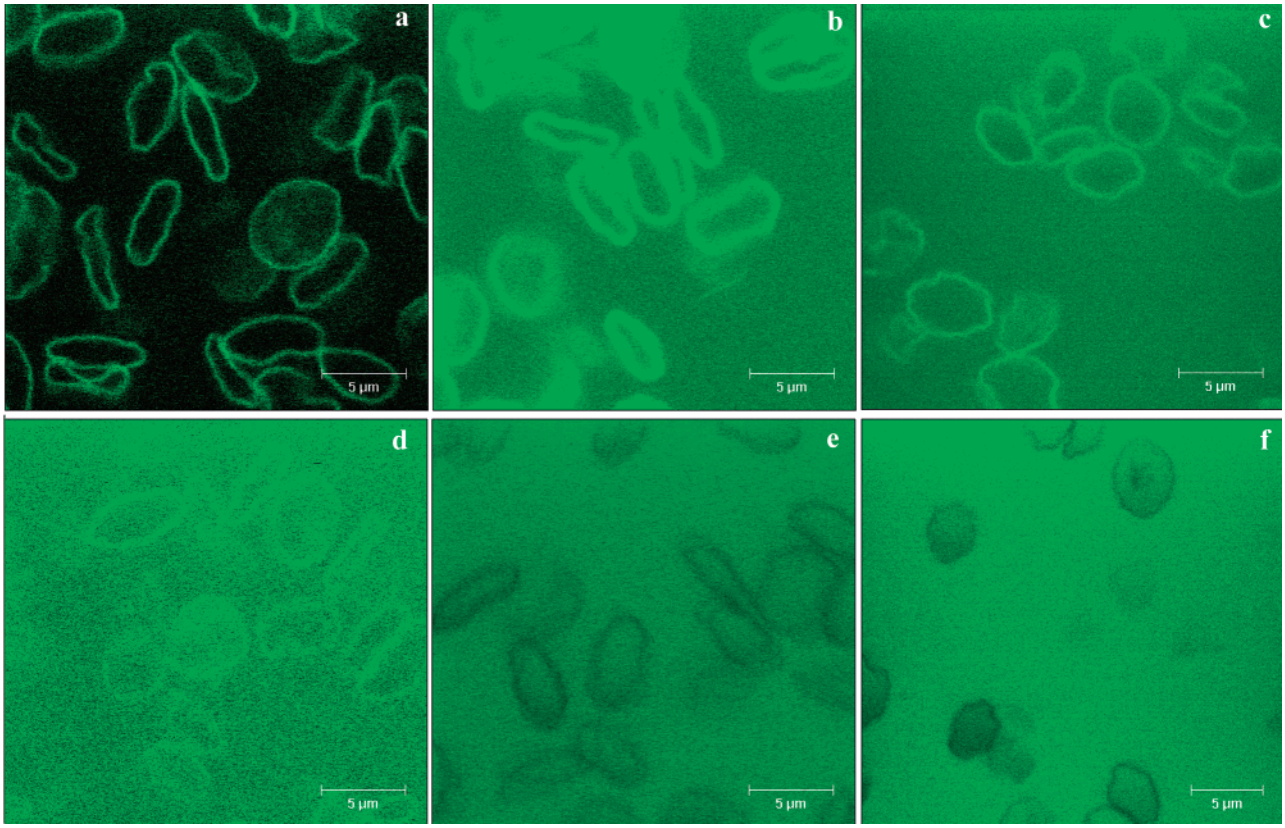

Figure 2. Confocal images of $(PSS/PAH)_2PSS$ capsules in solutions of 1 mg/mL FITC-HSA in 150 mM NaCl/borate buffer pH 4 (a, d), pH 6.5 (b, e), and pH 9 (c, f). Pictures d, e, and f show capsules preincubated with unlabeled HSA 1 mg/mL. All images were taken with the same laser intensity and 20 min after addition of the FITC-labeled albumin.

mobility of the bare template. The pH value has only a weak influence on the mobility of the capsules, and the charge is clearly negative for all samples.

The charge of the albumin is negative at the pH value of 6.5 ± 0.1 corresponding to our adsorption conditions in the NaCl−albumin solutions.

Thus, under the conditions of the experiments illustrated in Figure 1 it could be expected that albumin forms a monolayer on the PEMC surface.

The high fluorescence intensity of the wall in electrolyte solutions superimposes on the fluorescence from the interior. This is disadvantageous for permeability measurements. To decrease the adsorption of FITC-labeled albumin on the capsule wall, the suspension was first incubated with unlabeled HSA. Figure 2 compares images of preincubated samples with the control at a salt concentration of 150 mM for three pH values.

For PEMCs at pH 4 without preincubation, Figure 2a, the fluorescence is localized only at the capsule wall and the interior is dark as well as the bulk. This is a result of the strong adsorption due to the electrostatic attraction between the negatively charged capsule surface and the albumin molecules, which are positively charged at this pH value. At the albumin concentration used, almost no labeled protein remains in solution. The charge of albumin changes with increasing pH resulting in a lower adsorption of the protein as shown in the same figure for pH 6.5 (Figure 2b) and pH 9 (Figure 2c). The fluorescence of the background in these two cases indicates a relatively high concentration of protein in the solution. It has to be underlined that the same concentration of labeled albumin was employed in all three images.

As shown in Figure 2d preincubated capsules at pH 4 show a higher fluorescence coming from the walls, but in this case, the bulk solution and the capsule interior fluoresce with equal intensity.

At a pH of 6.5 the control, Figure 2b, shows higher fluorescence of the capsule walls than the bulk solution and the capsule interior. Preincubated PEMCs, Figure 2e, show no accumulation of fluorescence coming from the capsule wall. Indeed, a decrease in the fluorescence intensity takes place at the walls. The intensity of the bulk and capsule

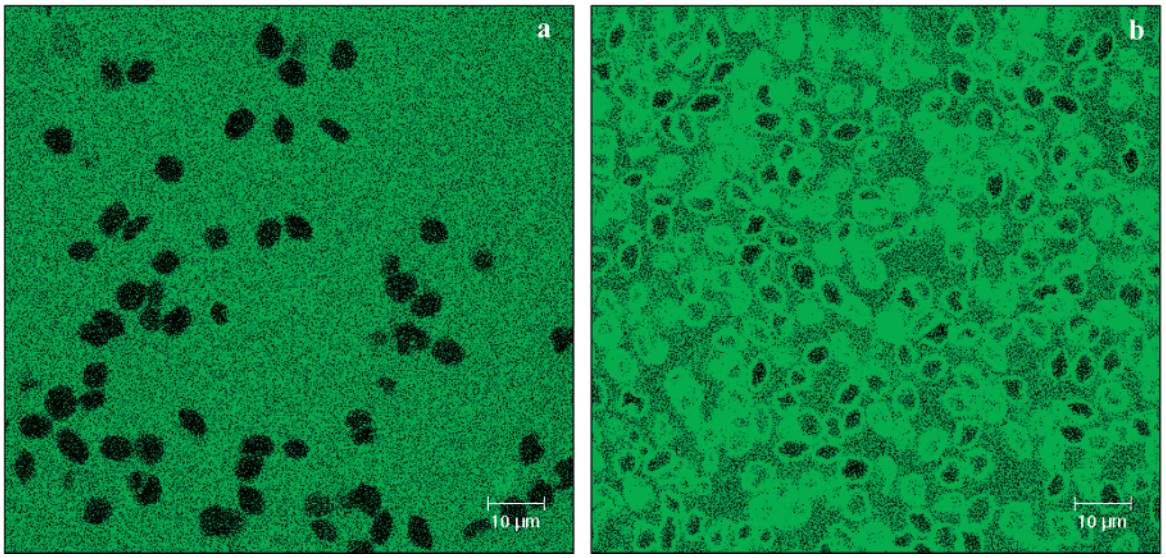

Figure 3. CLSM images of $(DxSO_4/PAH)_2DxSO_4$ capsules after incubation at room temperature for (a) 6 min and (b) 30 min in 1 mg/mL FITC-HSA in water.

Table 2. Relative Fluorescence Intensities Measured from the Images in Figure 2[a]

	control		preincubated PEMC	
pH	rel interior intens, I_i/I_e	rel wall intens, I_w/I_e	rel interior intens, I_i/I_e	rel wall intens, I_w/I_e
4	1 ± 0.22	5 ± 0.28	0.92 ± 0.15	1.3 ± 0.11
6.5	0.96 ± 0.14	1.5 ± 0.13	0.92 ± 0.12	0.7 ± 0.08
9	0.96 ± 0.15	1.1 ± 0.12	0.87 ± 0.14	0.7 ± 0.09

[a] The intensities of capsule interior and capsule wall are related to the fluorescence intensity of the bulk solution.

interior is equal. At pH 9 the permeability behavior of the control, Figure 2c, and the preincubated PEMC, Figure 2f, is the same. Although no visible adsorption of FITC-albumin takes place on the capsule wall, the permeation is not affected.

In Table 2 the fluorescence intensities of the capsule interiors and of the walls are related to the fluorescence intensity of the bulk for PEMCs incubated under the conditions described in Figure 2. It can be seen that the intensities of the capsule walls decrease after preincubation with unlabeled albumin more than 3 times at pH 4 and around 2 times for pH 6.5 and 9. In contrast the relative intensities of the capsule interior are approximately constant for all pH values and are not significantly different for the control and preincubated PEMCs.

The variation of the pH value of the solution proved that the permeability of the PEMCs was not dependent on pH. It follows from Figure 2 that HSA permeates at any pH when the salt concentration is large enough. Sukhorukov et al.[7] proposed a pH dependence of the permeability of macromolecules through the walls of melamine capsules. The lack of response of the RBC capsule walls to pH may be related to the different chemical structure of this capsules, where no amino or pH dependent groups are present.[15]

PEMCs made from $DxSO_4$/PAH and PSS/PAH were incubated with FITC−albumin for hours at room temperature in order to prove the stability of the "closed" state in water. Figure 3 illustrates the process at two different incubation

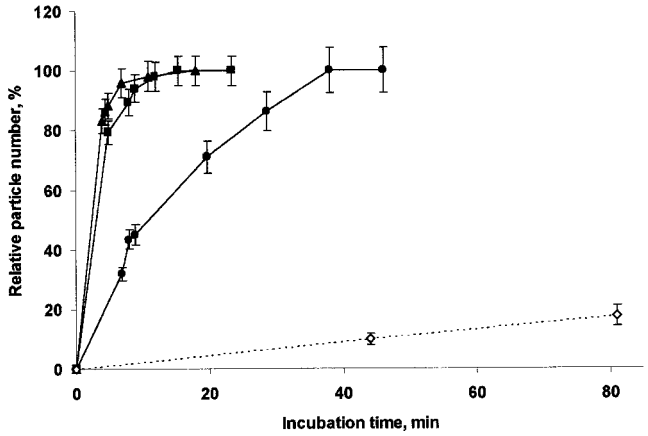

Figure 4. Percentage of filled $(DxSO_4/PAH)_2DxSO_4$ capsules after incubation in 1 mg/mL FITC-HSA solutions with time in water (solid circles), in 0.5 mM NaCl (solid squares), and in 1.5 mM NaCl (solid triangles). The open diamonds and the dashed line represent the percentage of filled $(PSS/PAH)_2PSS$ capsules with time in 0.15 mM NaCl.

times for $(DxSO_4/PAH)_2DxSO_4$ capsules. It can be seen that after 30 min the walls exhibit a strong fluorescence and most PEMCs show a fluorescence intensity of the interior equal to or higher than the fluorescence in the bulk. $(PSS/PAH)_2PSS$ capsules showed a similar behavior, but the process was much slower.

The effect of salt on the permeability of HSA through the capsule wall was studied in greater detail. PEMCs were observed with the confocal microscope at different times of incubation with FITC-HSA and in different salt concentrations.

In Figure 4, the percentage of $DxSO_4$/PAH−PEMC with FITC-HSA inside is plotted against time for various salt concentrations. For comparison one curve represents the uptake of PSS/PAH−PEMC at a low salt concentration (0.15 mM NaCl). The time dependence was followed up to 100% loading of the PEMCs.

In water the uptake of HSA into the $DxSO_4$/PAH−PEMC is slow. It levels off after about of 40−60 min. With

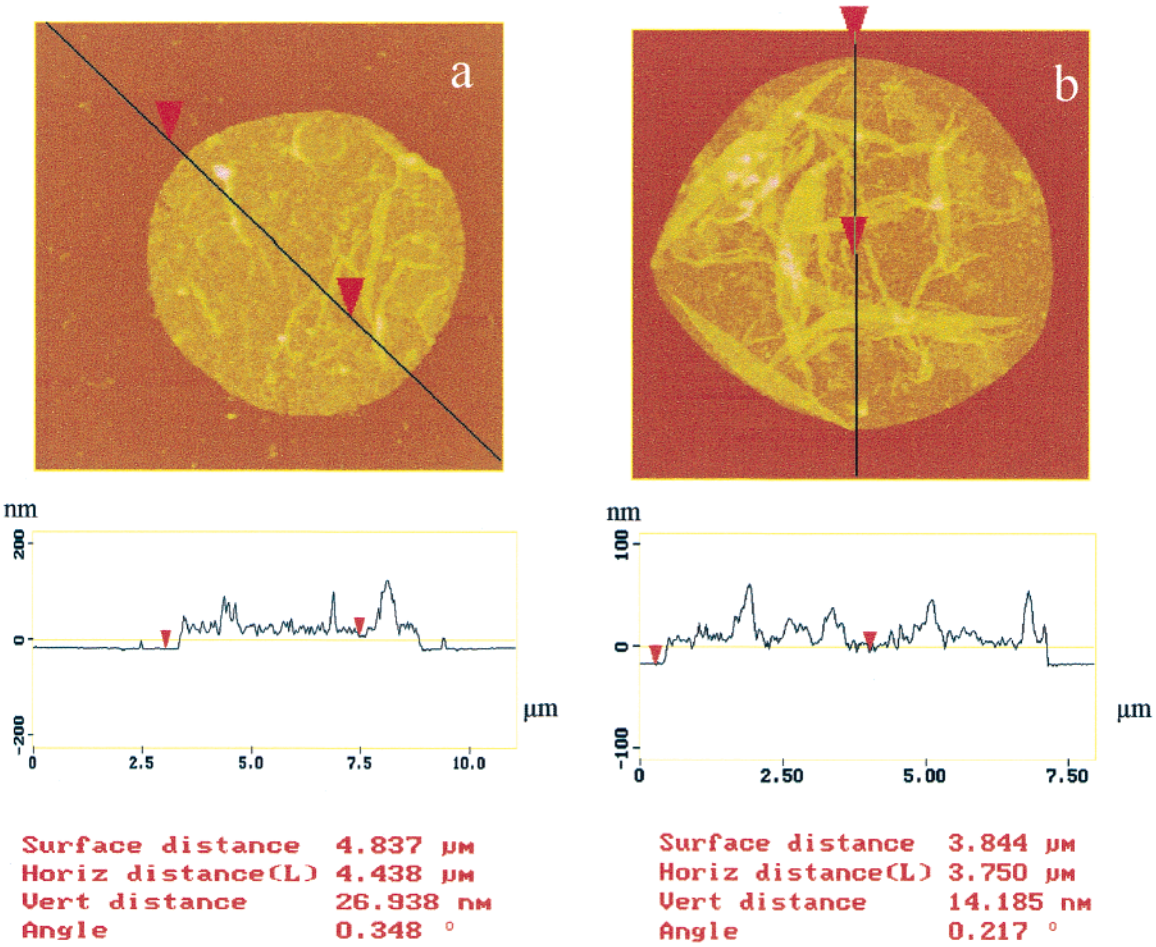

Figure 5. (top) SFM contact mode image of a RBC-templated $(PSS/PAH)_2PSS$ capsule (a) and $(DxSO_4/PAH)_2DxSO_4$ capsule (b). (bottom) Height profiles of the sections as indicated by arrows.

increasing salt concentration the uptake of HSA proceeds much faster. For example, at 1.5 mM NaCl within 10 min the uptake is completed.

The uptake of HSA into PSS/PAH capsules is generally slower. In water, loading can be achieved after a long period of about 10−12 h. At a concentration of 1.5 mM NaCl the uptake was completed after about 4 h, which was many times more than the loading time for $DxSO_4$/PAH capsules at the same salt concentration. The limiting salt concentration for a fast uptake in this case was 5 mM NaCl.

It is remarkable that PEMCs fabricated with PSS show a much longer characteristic time of uptake than PEMCs built up with $DxSO_4$. A different structure or a different thickness of the capsule wall can be the reason for the difference in permeability. In fact, the thickness of the polyelectrolyte walls is around 15 nm for the $(PSS/PAH)_2PSS$ and about 7 nm for the $(DxSO_4/PAH)_2DxSO_4$ capsules, as measured with SFM in dry state (Figure 5). The thickness of capsule walls was taken as half of the vertical distance measured between the mica support and the minimal height of the collapsed capsule.

The loading of the capsules with HSA after a longer time of about 1 h for $DxSO_4$/PAH and up to 12 h for PSS/PAH capsules in absence of or at low concentration of ions may be caused by defects in the wall occurring during incubation. Such an assumption is supported by the fact that the fluorescence coming from the capsule interior is quite inhomogeneous with regard to the population. Some are strongly fluorescent while others are dark at the same time. It is not clear how such defects appear. The consequence for the HSA permeation is that the uptake occurs stepwise. For a certain period of time a capsule remains impermeable. However, after the occurrence of a defect permeation quickly fills the capsule. In such a situation an average permeability is not correlating to a loading time of hours.

It may also be that adsorption itself induces after a while permeation of the charged HSA in the absence of salt or at very low ionic strengths. The adsorbed proteins interact with the polymer capsule wall, and this interaction may change the structure of the wall. This hypothesis is supported by the observation of many PEMCs with bright walls but dark interior shortly after HSA addition. The amount of these PEMCs decreases and the percentage of the loaded PEMCs increases with the time of incubation. It is further remarkable that in water the fluorescence in the interior is larger than that in the bulk, especially after incubation for larger periods of time. Since it is highly unlikely that the HSA is accumulated inside, this observation is currently related to a pH difference between the interior and the bulk brought about by some remains in the interior or at the inner surface, which buffer the capsule interior. The pH difference would result in a different fluorescence yield at equal HSA concentration. Studies are under progress to verify this hypothesis.

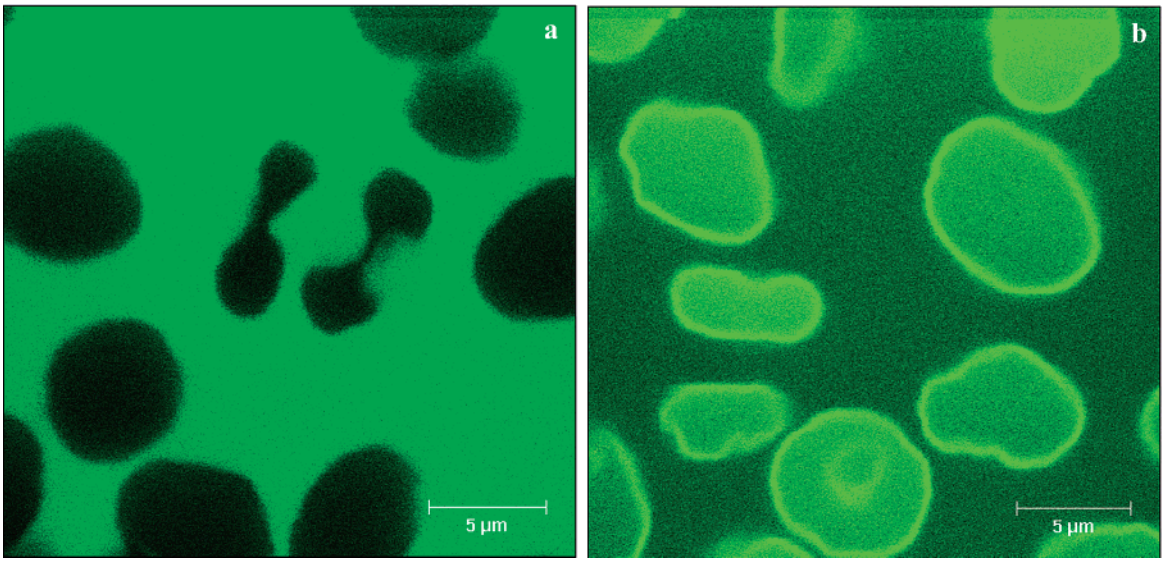

Figure 6. CLSM images of $(PSS/PAH)_7PSS$ incubated for 20 min in (a) 1 mg/mL FITC-HSA/water and (b) 1 mg/mL FITC-dextran M_w 77 kD/water.

Generally, the salt effect has to be related to the electrostatic interaction of the HSA with the capsule walls. To verify this assumption concerning the mechanism of permeation, the permeability of the capsule walls was studied for neutral polymers. As the permeating species, FITC-labeled dextran was employed. The labeling with FITC induced, however, a small negative charge of the dextran equal to one negative charge for every 90 glucose rings.

The confocal images in Figure 6 show $(PSS/PAH)_7PSS$ capsules suspended in solutions of (a) FITC-labeled HSA (66 kD) and (b) FITC-labeled dextran (77 kD), respectively, both in the absence of salt. The images were taken 20 min after addition of the labeled compounds. While the interior of the PEMC in the presence of FITC-dextran fluoresces strongly, FITC-HSA as demonstrated above did not permeate within this period of time. In contrast to HSA, which needed several hours for complete loading in the absence of salt, dextran permeates to the PEMC within the time of preparation of approximately 10 min. The hydrodynamic radius of the dextran molecules is comparable to that of albumin. HSA represents an ellipsoid with major and minor axes of 12.0 and 2.7 nm, respectively.[26] Dextran with a molecular weight of 74 kD has a gyration radius of 7.4 nm and an end-to-end distance of 14.9 nm.[27] Hence, the permeation of dextran through the capsule wall cannot be explained by a smaller molecular size. Electrostatic interactions have to be taken into account when comparing the very different permeability of dextran and HSA. It has also been shown above that HSA quickly permeates into the PEMC in the presence of salt. From these observations the following explanation is likely. Probably there are pores or defects present in the capsule wall, otherwise the dextran permeation is difficult to understand. These pores may be a consequence of the strong oxidation during preparation. They may have been stabilized or enlarged as a consequence of the osmotic swelling of the capsules during the dissolution and release process. Since the capsule wall is charged, electrostatic interactions of the permeating species with the pore surface certainly play a role. In the presence of salt the electrostatic interaction is weaker and charged molecules, as do the neutral ones, can pass more easily into the capsule interior.

The existence of pores should result in a cutoff of permeability with increasing molecular weight of dextran. Studies are under progress to demonstrate this.

The permeation of charged molecules would be affected to a lesser degree by the electrostatic interactions if they are much smaller than the pore size. This is the case with 6-carboxyfluorescein.[11] If the size of the molecule is comparable to the size of the pore, electrical interactions as well as blocking the pore due to adsorption may determine the permeability.

A control experiment with a polyelectrolyte of a charge opposite to the wall cannot be performed. Such a species will adsorb forming a new layer on the capsule surface changing its charge. This was for example the case with PAH.

Conclusions

The concentration of salt controls the permeation of HSA into PEMCs fabricated by means of templating red blood cells. At salt concentrations higher than 1 mM $DxSO_4/PAH$ capsules are highly permeable for HSA. For PSS/PAH capsules this limiting salt concentration is 5 mM. In water, loading of PEMC can be achieved only after many hours of incubation. The permeability depends on the capsule wall composition. The permeability of $(PSS/PAH)_2PSS$ capsules is at least 10 times smaller than that of $(DxSO_4/PAH)_2DxSO_4$ capsules. The permeability did not depend on pH.

The permeability of PEMC can be explained by the presence of pores or defects in the capsule wall generated during preparation and by the electrostatic interactions between the permeating molecules and the charged surface of the capsule. This is confirmed by the considerable increase of permeation of charged molecules in the presence of salt. Neutral molecules permeate regardless of the ionic strength.

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