Adhesion of Polyelectrolyte Microcapsules through Biotin−Streptavidin Specific Interaction

A. M. Raichur,[†,§] J. Vörös,[‡] M. Textor,[‡] and A. Fery*[,†]

Max Planck Institute for Colloids and Interfaces, Golm 14476 Germany, and BioInterphaseGroup, Laboratory for Surface Science and Technology, Department of Materials, ETH Zurich, CH-8093 Zurich, Switzerland

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Adhesion of PAH/PSS and PDADMAC/PSS capsules through electrostatic and specific interactions has been investigated using reflective interference contrast microscopy (RICM). Adhesion of capsules via electrostatic interactions was found to be spontaneous and strong. Capsules functionalized with poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) did not exhibit significant adhesion (as determined by the adhesion area) to streptavidin-coated substrates, whereas capsules functionalized with biotinylated PLL-*g*-PEG showed a significantly larger adhesion area. Using continuum mechanical models, the total adhesion energies for these cases were calculated and were found to correspond to several tens of individual biotin-streptavidin pairs. The application of specific interactions such as the biotin−streptavidin system for controlled capsule adhesion has been demonstrated in this study.

Introduction

In recent years, preparation of micron- to submicron-sized polyelectrolyte capsules has received considerable attention.[1] The interest in these microcapsules arises due to their potential application as microreservoirs in several important areas such as medicine for drug delivery,[2] paper and textile coatings, encapsulating flavors (food design), and perfumes (cosmetics).[3] These hollow shells can also be used for mimicking biological cells. It is well-known that biological cells are very complex microcapsules due to their complex cell wall structure and varying surface chemical composition[4] which makes understanding of their adhesion difficult. However, by using polyelectrolyte capsules, one can change the surface chemistry systematically and use them as model systems for mimicking biological cells. For example, the composition of the shells can be chosen among a wide variety of materials.[5,6] The thickness of the shell wall can be chosen and varied in nanometer-sized steps without affecting the adhesion properties which are determined by the last layer.[7] The diameter also can be chosen as desired varying from 100 nm to several microns.[8]

The polymeric shells can be made by various techniques of which the layer-by-layer (LbL) method is the most widely used.[9] Polyelectrolytes are coated on to a variety of templates including melamine formaldehyde,[6] polystyrene latex,[7] $MnCO_3$,[10] $CaCO_3$,[11] biological cells,[12] and protein aggregates.[13] Depending on the type of polyelectrolyte used, the thickness of the shell wall, the surface charge, and the surface hydrophobicity can be varied which in turn determine the adhesive properties of the capsules. Since the surface of capsules is naturally charged, electrostatic interactions can bring about adhesion to oppositely charged surfaces. In previous work,[14] it has already been shown that the adhesion properties of PSS/PAH capsules through electrostatic interactions strongly depends on the capsule wall thickness. The coupling of capsules on patterned surfaces through electrostatic interactions has also been demonstrated.[15] Although electrostatic interactions are strong, they are nonspecific. For application in drug delivery systems, the capsules interact and target specific cells, tissues, and organs. Therefore, the surface property of the capsules needs to be tailored such that only the desired interaction takes place. Such specificity is achieved by using key-lock type interactions, and one of the strongest interactions of this type is the biotin−streptavidin specific interaction, which can therefore serve as a model for specific functionalization of microcapsules. The application of this approach for producing microcapsule arrays has been shown recently.[16]

Surface functionalization of biomaterials such as TiO_2 and poly(lactic-*co*-glycolic acid) (PLGA) microspheres using a poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) coating have been used with the aim of preventing adsorption of proteins.[17−19] The polycationic graft copolymer adsorbs as a monolayer to negatively charged surfaces, forming a brush of hydrated PEG chains that resists protein adsorption by excluded volume effect, steric repulsion, and screening of interfacial charges.[20−21] PLL-*g*-PEG molecule with a fraction of the PEG side chains functionalized with biotin has also been developed and applied to bioaffinity sensing.[22] In another study, PLL-*g*-PEG/PEG-biotin has been shown to adsorb spontaneously on to PSS/PAH capsules[23] thus producing biofunctionalized capsules.

In this study, we present the application of PLL-*g*-PEG-biotin to produce biofunctionalized poly(allylamine hydrochloride)/poly(styrene sulfonate) (PAH/PSS) and poly(diallyldimethylammonium)/poly(styrene sulfonate) (PDADMAC/PSS) capsules with an electrically neutral surface presenting biotin ligands on a noninteractive ("nonfouling") background. Adhesion of such capsules on to streptavidin-coated glass substrates was studied by reflective interference contrast microscopy (RICM). Based on the adhesion areas obtained, the adhesion energies were estimated for specific interactions. As a control, adhesion of

* Corresponding author. E-mail: andreas.fery@mpikg.mpg.de.
† Max Planck Institute for Colloids and Interfaces.
‡ ETH Zurich.
§ On sabbatical leave from: Department of Metallurgy, Indian Institute of Science, Bangalore 560012 India.

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nonbiotinylated PLL-*g*-PEG coated capsules and PSS terminated capsules was also tested.

Materials and Methods

Capsules. $[PAH/PSS]_8$ and $[PDADMAC/PSS]_{10}$ capsules were prepared and supplied by Capsulution Nanoscience AG, Berlin, Germany and were then characterized in our laboratory. Briefly, the PAH/PSS capsules were prepared using 7.2 μm diameter melamine formaldehyde template at pH 5.6 and dissolved at around pH 2. The polyelectrolyte concentration was 1 mg/mL in 0.5 M NaCl solution. PDADMAC/PSS capsules were prepared with 4.7 μm silica templates at pH 5.6 using 1 mg/mL polyelectrolyte in 0.5 M NaCl solution. The templates were dissolved around pH 2.3 using 1 M HF solution. Rhodamine-labeled PSS was used to prepare capsules, and optical images of the capsules were obtained using a Leica TCS SP confocal system (Leica, Germany). The last layer of the as-received capsules was always PSS and was supplied at 1% v/v concentration in water.

PLL-*g*-PEG and PLL-*g*-PEG-Biotin Molecules. PLL-*g*-PEG and PLL-*g*-PEG-biotin (biotin density 15%) were supplied by SurfaceSolutionS GmbH, Zurich, Switzerland, and streptavidin (mol wt. 53000) was purchased from Invitrogen GmbH, Karlsruhe, Germany and were all stored at −20 °C. PLL-*g*-PEG and PLL-*g*-PEG-biotin solutions were prepared in 10 mM HEPES (Sigma, Seelze, Germany) buffer and adjusted to pH 7.2 using 3 M NaOH (E. Merck, Darmstadt, Germany). Streptavidin was dissolved in 10 mM HEPES plus 150 mM NaCl (E. Merck, Darmstadt, Germany) adjusted to pH 7.2. The solutions were refrigerated and were used for a period of 2−3 weeks. The chemical structures of PLL-*g*-PEG and PLL-*g*-PEG-biotin are given in Figure 1. The molecular weight of PLL was 20 kDa whereas the PEG side chain was 2 kDa and the grafting ratio (*g*) was 3.5 which represent the ratio of lysine units to PEG units.

Zeta-Potential Measurements. The surface charge of the capsules (with and without coating with PLL-*g*-PEG) was measured using a Malvern Zetasizer 3000 HS. A small amount of as-received capsules was suspended in 10 mM HEPES buffer. For preparing coated capsules, 100 μL of PSS terminated capsules were suspended in 250 μL of 0.1 mg/mL PLL-*g*-PEG and PLL-*g*-PEG-biotin solutions at pH 7.2. Coating was done for 1 h at room temperature with intermitting stirring. The capsules were centrifuged at 400 rcf for 25 min. The capsules were washed twice to remove the excess polymer solution, and the coated capsules were resuspended back in 6 mL of 10 mM HEPES buffer. About 10 zeta potential readings were taken for each sample and averaged. Selected samples were also analyzed with the Malvern Nano Zetasizer to cross check the results.

AFM Imaging. The thickness and quality of the capsules' membranes were studied using AFM. Imaging of dried capsules was done in tapping mode using the Dimension 3000 AFM (Nanoscope III controller). The SiN cantilevers (Nanoworld Services GmbH, Erlangen, Germany) had the following characteristics: type NCR−W; resonance frequency ∼ 300 kHz; spring constant ∼ 42 N/m. Background corrections to the images were done by flattening and wall thicknesses were determined using the SPIP software.

Adhesion Studies. Adhesion of capsules on glass substrates was studied by RICM on a Zeiss Axiovert 200 microscope. For electrostatic interaction the glass substrate was first coated with polyethylenimine (PEI) (molecular weight 750 000; Sigma, Seelze, Germany), whereas for specific interactions, the substrate was coated with streptavidin. The substrates (cover slips; 24 × 24 mm) were first cleaned by RCA method[24] and dried under nitrogen. A few drops of PEI (0.6 mg/mL) or streptavidin (0.1 mg/mL) were placed on the cover slip. After about 30 min, the substrates were rinsed thoroughly with distilled water and dried under nitrogen before use. Capsules were coated by adding 20 μL of PSS terminated capsules to 100 μL of PLL-*g*-PEG or PLL-*g*-PEG-biotin solution and allowed to adsorb for 1 h. The capsules were then centrifuged, washed and resuspended in 100 μL HEPES buffer before use.

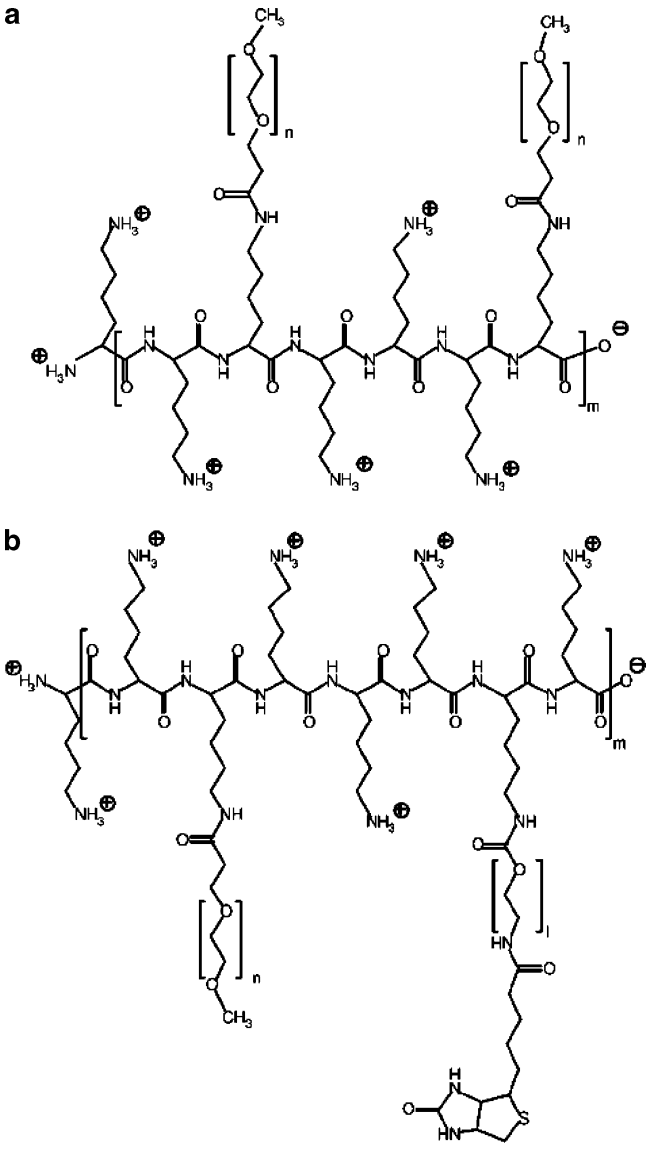


Figure 1. (a) Structure of PLL-*g*-PEG. (b) Structure of PLL-*g*-PEG biotin.

A Zeiss Antiflex 63xNO oil immersion objective with polarizers was used for RICM. About 20 μL of capsule suspension was placed on the substrate, and capsules were allowed to interact and adhere to the substrate (typically 20−25 min). The adhered capsules produced a fringe pattern which was captured using the Zeiss AxiocamHR camera and Zeiss Axiovision software. Generally about 30−40 capsules were imaged, and the adhesion area was determined. The effect of electrostatic interaction and specific interactions between PLL-*g*-PEG/PLL-*g*-PEG-biotin coated capsules with streptavidin coated glass on the adhesion of capsules was investigated.

In RICM, the sample is illuminated with monochromatic light in reflection geometry. Depending on the local distance between the capsule wall and the glass surface, the reflected light will interfere constructively or destructively.[25,26] A pattern of alternating dark and bright fringes is thus obtained with a dark portion in the center. This part represents the area of the capsule that is closest to the glass surface. In cases where a several micron sized contact area between microcapsule and surface is established, it is easily identified as a region of constant gray level, which is surrounded by several concentric fringes. This type of behavior was observed in earlier studies and here for electrostatically triggered adhesion, and Figure 5a displays a typical example of such a strong adhesion situation. If interactions are weaker such that only a submicron contact area forms between microcapsule

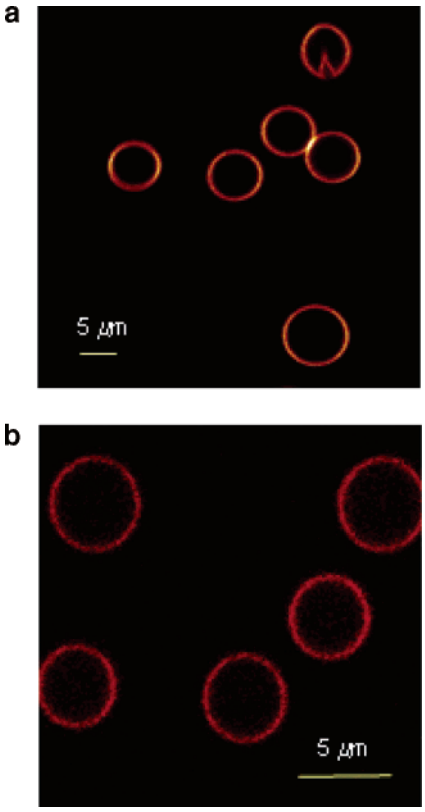


Figure 2. Confocal images of (a) PAH/PSS capsules and (b) PDADMAC/PSS capsules.

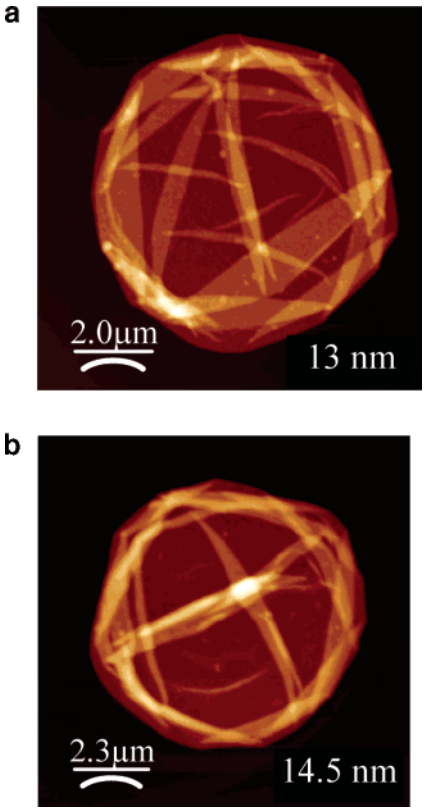


Figure 3. AFM images of dried PAH/PSS capsules (a) PSS terminated and (b) PLL-*g*-PEG coated.

and surface, a straightforward interpretation of the interference pattern in terms of contact areas is not possible. A quantitative determination of the adhesion area in such cases would require knowledge of the lights phase shift upon reflection from the capsule, which is not

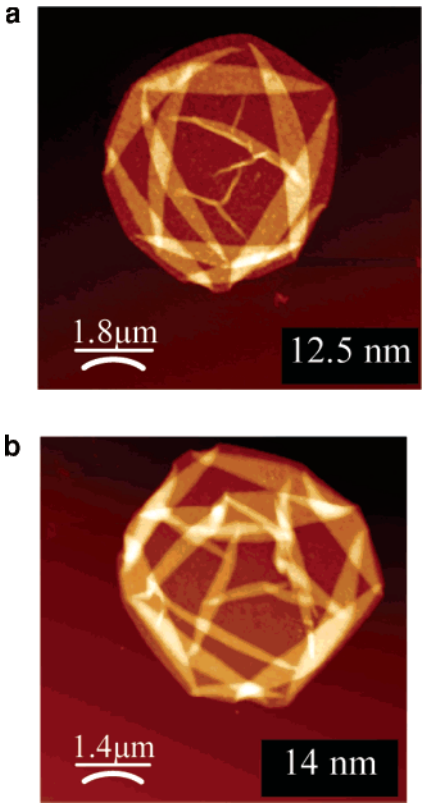


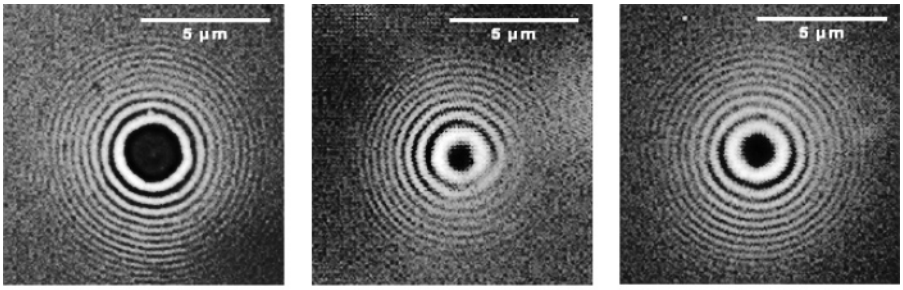
Figure 4. AFM images of dried PDADMAC/PSS capsules (a) PSS terminated and (b) PLL-*g*-PEG coated.

accessible. Still, for membranes of identical reflectivity, the radius of the area up to the first bright fringe can be used as a measure of adhesion strength, and we have used this "apparent adhesion area" for comparison of adhesion strength. The apparent adhesion area is determined as follows. About 20−30 fringe patterns of the same pixel size are opened as an image sequence in the Image-J software and the contrast is enhanced by normalizing and equalizing the histograms. The scaling is then set for the appropriate magnification and objective. The threshold for the entire image sequence is then set to a particular value so that the center dark portion of the fringe pattern is covered. This value will be the same for all of the patterns analyzed under various conditions. Finally, using the wand and measure functions, the center portion depicting the apparent adhesion area is determined for the entire image sequence.

Results and Discussion

Characterization of Capsules. The as-received PAH/PSS and PDADMAC/PSS capsules were first characterized for their size and mono-dispersity by confocal microscopy (Figure 2). As can be seen, the capsules were monosized (7.2 μm for PAH/PSS and 4.7 μm for PDADMAC/PSS) and spherical in shape. AFM images (Figures 3 and 4) showed that the capsules did not have any template residue inside or entrapped in the wall. The wall thickness of the PAH/PSS and PDADMAC/PSS capsules as determined by AFM was about 13 and 12.5 nm (for PSS terminated capsules) and 14.5 and 14 nm (for PLL-*g*-PEG coated capsules), respectively. The small increase in the wall thickness shows that PLL-*g*-PEG has been coated on the capsules. The typical dry thickness of PLL-*g*-PEG coatings is 1.5 nm.[21]

Zeta-potential values of the capsules under different conditions were measured and are tabulated below.The zeta potential of the PSS terminated capsules was about −50 mV for both



(a) Electrostatic 3.33 (±0.6) μm^2 (b) PLL-*g*-PEG 0.85 (±0.16) μm^2 (c) PLL-*g*-PEG-biotin 1.21 (±0.15) μm^2

Figure 5. RICM images showing the apparent adhesion areas of PAH/PSS capsules with different outer layers. (a) Electrostatic 3.33 (±0.6) μm^2, (b) PLL-*g*-PEG 0.85 (±0.16) μm^2, and (c) PLL-*g*-PEG-biotin 1.21 (±0.15) μm^2.

Table 1. Zeta Potential Values of the Two Capsule Systems with Different Outer Layer Coatings

	zeta potential (mV)		
sample	PSS terminated	PLL-*g*-PEG coated	PLL-*g*-PEG-biotin coated
PAH/PSS	−51.1 (±1.1)	−0.3 (±0.8)	0.2 (±1.3)
PDADMAC/PSS	−50.4 (±0.9)	0.2 (±1.2)	0.8 (±0.95)

types of capsules. However, for the PLL-*g*-PEG and PLL-*g*-PEG-biotin coated capsules, the charge was significantly reduced to almost zero for both capsule systems. This reduction in charge to near neutral values indicates that the capsules are coated with the PLL-*g*-PEG molecule since the negative charge of the PSS-terminated capsules is almost exactly compensated by the positive charge of the "free" non-PEGylated amine groups of the PLL backbone. Adsorption kinetics of PLL-*g*-PEG on to the capsules showed that after 20 min incubation the surface was neutralized indicating complete adsorption. These results are consistent with those reported earlier[23] for PSS/PAH capsules.

Adhesion Studies. Adhesion of capsules was studied by determining the apparent adhesion area of capsules on flat coated glass substrates. A typical set of images obtained by RICM for PAH/PSS and PDADMAC/PSS capsules is given in Figures 5 and 6, respectively. Figures 5a and 6a show the fringe pattern obtained when a PSS terminated capsule adheres to a PEI coated substrate and the apparent adhesion area was calculated to be about 3.33 μm^2 for PAH/PSS capsule and 1.33 μm^2 for PDADMAC/PSS capsule. Adhesion took place within minutes and was found to be strong and irreversible. The irreversibility of adhesion was determined by stirring the solution on the glass substrate using a micropipet. None of the capsules were detached from the glass substrate. This observation is consistent with results obtained in earlier studies[14,15] that indicate strong adhesion.

When the capsules were coated with PLL-*g*-PEG and allowed to interact with streptavidin-coated substrates, the interaction reduced significantly and the apparent adhesion area decreased to about 0.85 and 0.4 μm^2 for PAH/PSS and PDADMAC/PSS capsules respectively (see Figures 5b and 6b). It took about 45−75 min before the capsules came close to the glass surface and took another 10−15 min before Brownian motion of the capsules stopped indicating contact formation. This indicates that there are only weak interactions between PLL-*g*-PEG and streptavidin probably due to entanglement. This contact area could be comparable with a pointlike contact between the shell and the fuzzy surface. The adsorption of streptavidin on the glass substrate was confirmed by a separate test where fluorescently labeled streptavidin was coated and observed under a fluorescence optical microscope (data not shown). Therefore, it can be inferred from this experiment that PLL-*g*-PEG coated capsules do not interact strongly with any kind of surface since they carry almost no charge and do not present any functional, interactive group.

This is confirmed in the next set of experiments where PLL-*g*-PEG-biotin coated capsules were allowed to interact with streptavidin-coated substrates. In this case, adhesion kinetics was faster (about 30−45 min before capsules stopped Brownian motion) but slower compared to electrostatic interaction. This could be due to the fact that electrostatic forces are much stronger which leads to rapid adhesion. However, in specific interactions such as the key−lock biotin−streptavidin interaction, the biotin has to approach the streptavidin molecule before the attachment takes place. Therefore, interaction is dependent on the settling rate of the capsule and the probability of the biotin molecule finding a streptavidin site.

The capsules formed fringe patterns as shown in the Figure 5c and 6c and the apparent adhesion areas were found to be around 1.21 and 1.14 μm^2 for PAH/PSS and PDADMAC/PSS systems. As demonstrated by the zeta-potential measurements, the surface charge on the capsules is close to zero. The only interaction here is the biotin−streptavidin interaction. Therefore adhesion must occur in this case through specific interactions. More over, the adhesion of capsules can be altered by changing the surface coating. The large apparent adhesion area obtained through electrostatic interactions is minimized when no interaction is present between the capsule and substrate. However, when specific interactions are present, the apparent adhesion area is again observed although it is smaller compared to electrostatic interactions. When the suspension was stirred after the capsules had settled, all of the capsules detached off from the surface indicating that specific interactions are weak in nature compared to electrostatic interactions.

Experimentally, we were able to observe that adhesion due to specific interactions was weak compared to that with electrostatic interactions. The adhesion energy for the PSS/PAH system through electrostatic interactions is about 0.28 mJ·m^{-2}.[14] Based on the approach of Elsner et al.[14] and using the model for adhesion energy estimation for thin capsules, we have estimated the adhesion energy for both types of capsules interacting through biotin-streptavidin reaction. For this calculation, we have assumed that the microcapsules form a pointlike adhesion area in the case of the PEG−streptavidin interaction. Thus, the problem of the unknown phase shift upon reflection on the microcapsule wall is eliminated and the area of adhesion

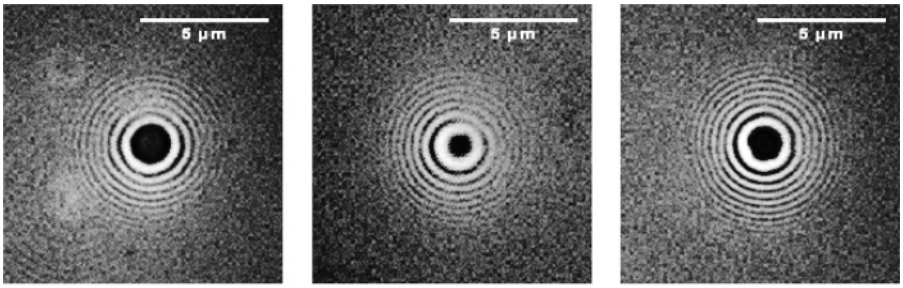


Figure 6. RICM images showing the apparent adhesion areas of PDADMAC/PSS capsules with different outer layers. (a) Electrostatic 1.33 (±0.45) μm^2, (b) PLL-*g*-PEG 0.40 (±0.12) μm^2, and (c) PLL-*g*-PEG-biotin 1.14 (±0.10) μm^2.

can be calculated for the cases of specific interactions. The corrected adhesion area for PAH/PSS is 0.36 μm^2, whereas for PDADMAC/PSS, it is 0.74 μm^2. The elastic moduli for PAH/PSS and PDADMAC/PSS capsules are 0.269[27] and 0.10 GPa,[28] respectively. The elastic modulus of PDADMAC/PSS shows a large variation due to swelling of these multilayers in water, and hence, the average value has been used here. Using the wall thickness shown in Figures 3b and 4b, the adhesion energies were estimated to be 6.628×10^{-6} J·m^{-2} for PAH/PSS and 16.33×10^{-6} J·m^{-2} for PDADMAC/PSS capsules. The adhesion energies through specific interactions are significantly lower compared to the electrostatic interactions[14,29] which corroborates with our experimental observation. The adhesion energies calculated can be taken as an upper estimate since there could be other interactions such as secondary interactions contributing to the adhesion energy.

Further the total energy for the contact area formed during the PEG−biotin and streptavidin interaction was calculated to be 2.38×10^{-18} J for PAH/PSS capsules and 1.21×10^{-17} J for PDADMAC/PSS capsules. The bond energy for a pair of biotin-streptavidin is about 35 kT[30] which at room temperature is 1.4×10^{-19} J per pair. From the total energy values, the number of biotin−streptavidin pairs interacting in the contact area works out to be 17 pairs for PAH/PSS capsules and 86 pairs for PDADMAC/PSS capsules. Thus, both systems are in a regime of multiple site interaction. The number of pairs interacting for the PDADMAC capsules is significantly higher even though the PDADMAC/PSS capsules are smaller (4.7 μm dia) compared to the PAH/PSS capsules (7.2 μm dia). The elastic modulus for PAH/PSS is higher compared to PDADMAC/PSS capsules which means that for the softer capsules, the biotin−streptavidin interaction is able to form a larger contact area as compared to the stiffer ones. Therefore, the capsule mechanics play a critical role in determining the adhesion area when capsules interact through specific interactions.

Thus, by changing the surface composition and type of interaction, the adhesion of capsules can be controlled. The application of specific interactions in controlling adhesion of capsules is clearly demonstrated and such specific recognition can be used to control capsule adhesion.

Summary and Conclusions

PLL-*g*-PEG and PLL-*g*-PEG-biotin could be successfully coated on to PAH/PSS and PDADMAC/PSS polyelectrolyte multilayer capsules. Both microcapsule systems differ in their Young's modulus, whereas their surface interactions after coating are expected to be identical. For both capsule types, negatively charged PSS-terminated capsules showed almost no charge after coating with the PLL-*graft*-PEG molecule in zeta potential measurements. The kinetics of adsorption was found to be rapid and the coated capsules were found to be stable over a period of at least 4 weeks. The adhesion behavior was found to be strongly influenced by the coating process. Uncoated (PSS terminated) microcapsules showed strong electrostatic interactions, which triggered irreversible adhesion on oppositely charged surfaces and circular adhesion areas were formed. The coated microcapsules' adhesion behavior indicated a dominant role of specific interactions, when the microcapsules were brought into contact with streptavidin coated surfaces. Nearly negligible adhesion was found when PLL-*g*-PEG coated capsules for both capsule systems. Since the surface charge has been neutralized/screened by the polycationic polymer, there is no other interaction present and hence only weak adhesion was noticed. However, when PLL-*g*-PEG-biotin capsules were interacted with streptavidin-coated glass substrates, significantly larger adhesion areas were observed. Using continuum mechanical models, we could calculate the total adhesion energy for these cases and find that it corresponds to several tens of individual biotin-streptavidin pairs. Notably, softer capsules showed a larger adhesion energy, which indicates that, the steric constraints which have to be satisfied for bond formation can be easier met for a more deformable system.

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References and Notes

(1) Decher, G. In *Multilayer thin films: Sequential assembly of nanocomposite materials*, 1st ed.; Decher, G., Schlenoff, B. J., Eds.; Wiley-VCH: Weinheim, Germany, 2003; pp 1−46.
(2) Park, K. *Controlled Drug Delivery: Challenges and Strategies*; American Chemical Society: Washington DC, 1997.
(3) Kreuter, J. *Colloidal Drug Delivery Systems*; Marcel Dekker: New York, 1994.
(4) Lipowsky, R.; Sackmann, E. *Structure and dynamics of membranes*; Elsevier: Amsterdam, 1995.

(5) Möhwald, H.; Donath, E.; Sukhorukov, G. B. In *Multilayer thin films: Sequential assembly of nanocomposite materials*, 1st ed.; Decher, G., Schlenoff, J. B., Eds.; Wiley-VCH: Weinheim, Germany, 2003; pp 363−392.
(6) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem. Int. Ed.* **1998**, *37*, 2202−2205.
(7) Sukhorukov, G. B.; Donath, E.; Lichtenfeld, H.; Knippel, E.; Knippel, M.; Budde, A.; Möhwald, H. *Colloids Surf. A-Physicochem. Eng. Aspects* **1998**, *137*, 253−266.
(8) Caruso, F.; Niikura, K.; Furlong, D. N.; Okahata, Y. *Langmuir* **1997**, *13*, 3422−3426.
(9) Peyratout, C. S.; Dähne, L. *Angew. Chem.* **2004**, *43*, 3762−3783.
(10) Déjugnat, C.; Sukhorukov, G. B. *Langmuir* **2004**, *20*, 7265−7269.
(11) Volodkin, D. V.; Larionova, N. I.; Sukhorukov, G. B. *Biomacromolecules* **2004**, *5*, 1962−1972.
(12) Neu, B.; Voigt, A.; Mitlöhner, R.; Leporatti, S.; Gao, C. Y.; Donath, E.; Kiesewetter, H.; Möhwald, H.; Meiselman, H. J.; Bäumler, H. *J. Microencapsul.* **2001**, *18*, 385−395.
(13) Volodkin, D. V.; Balabushevitch, N. G.; Sukhorukov, G. B.; Larionova, N. I. *Biochemistry (Moscow)* **2003**, *68*, 283−289.
(14) Elsner, N.; Dubreuil, F.; Fery, A. *Phys. Rev. E* **2004**, *69*, 031802.
(15) Nolte, M.; Fery, A. *Langmuir* **2004**, *20*, 2995−2998.
(16) Wang, B.; Zhao, Q.; Wang, F.; Gao, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1560−1563.
(17) Kenausis, G. L.; Vörös, J.; Elbert, D. L.; Huang, N. P.; Hofer, R.; Ruiz-Taylor, L.; Textor, M.; Hubbell, J. A.; Spencer, N. D. *J. Phys. Chem. B* **2000**, *104*, 3298−3309.
(18) Huang, N. P.; Michel, R.; Vörös, J.; Textor, M.; Hofer, R.; Rossi, A.; Elbert, D. L.; Hubbell, J. A.; Spencer, N. D. *Langmuir* **2001**, *17*, 489−498.
(19) Müller, M.; Vörös, J.; Csucs, G.; Walter, E.; Danuser, G.; Textor, M.; Spencer, N. D. *J. Biomed. Mater. Res. A* **2003**, *66*, 55−61.
(20) Michel, R.; Pasche, S.; Textor, M.; Castner, D. G. *Langmuir* **2005**, *21*, 12327−12332.
(21) Pasche, S.; Vörös, J.; Griesser, H. J.; Spencer, N. D.; Textor, M. *J. Phys. Chem. B* **2005**, *109*, 17545−17552.
(22) Huang, N. P.; Vörös, J.; De Paul, S. M.; Textor, M.; Spencer, N. D. *Langmuir* **2002**, *18*, 220−230.
(23) Heuberger, R.; Sukhorukov, G. B.; Vörös, J.; Textor, M.; Möhwald, H. *Adv. Funct. Mater.* **2005**, *15*, 357−366.
(24) Kern, W.; Puotinen, D. A. *RCA Rev.* **1970**, *31*, 187−206
(25) Wiegand, G.; Jaworek, T.; Wegner, G.; Sackmann, E. *J. Colloid Interface Sci.* **1997**, *196*, 299−312.
(26) Wiegand, G.; Neumaier, K. R.; Sackmann, E. *Appl. Opt.* **1998**, *37*, 6892−6905.
(27) Heuvingh, J.; Zappa, M.; Fery, A. *Langmuir* **2005**, *21*, 3165−3171.
(28) Gao, C.; Leporatti, S.; Moya, S.; Donath, E.; Möhwald, H. *Langmuir* **2001**, *17*, 3491−3495.
(29) Bosio, V.; Dubreuil, F.; Bogdanovic, G.; Fery, A. *Colloids Surf. A: Physicochem. Eng. Aspects* **2004**, *243*, 147−155.
(30) Wong, J. Y.; Kuhl, J. L.; Israelachvili, J. N.; Mullah, N.; Zalipsky, S. *Science* **1997**, *275*, 820−822.

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