NANO LETTERS

2007
Vol. 7, No. 11
3540−3546

Fusion of Enveloped Virus Nanoparticles with Polyelectrolyte-Supported Lipid Membranes for the Design of Bio/Nonbio Interfaces

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Received September 13, 2007; Revised Manuscript Received October 14, 2007

ABSTRACT

Fusion of lipid-enveloped viruses with endosomal membranes triggered by low pH in the endosome is a key step in the course of viral infection. This ubiquitous mechanism can be used to integrate functional nanoparticles of viral origin into composite materials consisting of a polyelectrolyte multilayer with an adsorbed lipid membrane in a natural and biomimetic way. Polyelectrolyte multilayers as the support for the lipid membrane are a versatile means to combine the biological functions of the viral surface with the multiplicity of polyelectrolyte borne functions into a novel bio/nonbio composite material.

Viruses find increasing application in materials science and technology as building blocks for composite materials.[1] Viruses are essentially nanoparticles, the surface of which can be tailored in many ways both by molecular biology techniques and bioconjugation chemistry. The use of virus particles as functional parts of devices requires adequate techniques for their stable integration into the otherwise nonbiological environment without interfering with the biological functions carried on their surface. A natural solution for this problem would be to take advantage of membrane fusion mimicking viral infection via the endosomal pathway.[2] As a result of this process the viral envelope together with their peptide-based functions would become firmly integrated into the lipid bilayer on the surface of the synthetic material.

The surface of viruses is equipped with proteins for recognition and interaction with the host cell. In the case of many lipid-enveloped viruses, infection involves fusion of the viral membrane with the endosomal membrane before the genome of the virus is released into the cytoplasm. The membrane fusion proteins of these viruses become activated by the acidic pH value within the endosome and fusion proceeds driven by membrane insertion of a hydrophobic peptide and subsequent conformational changes of the fusion proteins.[3] Enveloped eucaryotic viruses play an important role in life sciences, medicine, and technology. The development of virus surface display systems based on such viruses,[4] research on viruslike particles as vaccines,[5] as well as the development of viral vector systems for gene therapy[6] has led to a versatile pool of virus nanoparticles equipped with native but also engineered surface features. An important point is that regardless of surface modifications the production of virus particles in most cases is based on infectivity, rendering the fusion mechanism with lipid membranes a strongly preserved feature. Fusing such viral nanoparticles with lipid membranes on solid supports is a natural way for their integration into a biomimetic environment, because it is based on the built-in functions of the virus. The formation of supported lipid bilayers (SLBs)[7] when combined with virus fusion is thus a promising technology platform of

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10.1021/nl0723580 CCC: $37.00
Published on Web 10/26/2007

fabricating biocompatible and biofunctional surfaces. When polyelectrolyte multilayers[8] fabricated with Layer by Layer (LbL) technology are used as a support for the lipid membrane,[9] other (nonbiological) functions can be easily incorporated into the multilayer structure. For example, it has been shown that virus-equipped lipid-coated LbL colloids with graduated fluorescence[10] can be used as diagnostic devices in a multiplexed bead array.[11] The use of viruses, which had been modified by directed evolution toward the display of functional peptides, is a general means for transferring biological functions onto these composites.[12] However, in these demonstrations of the approach it has not been convincingly shown that indeed fusion has occurred and that fusion can be triggered by pH. In our opinion, the stable integration of the viral envelope into the lipid layer versus, for example, merely adsorption is a prerequisite for the use in devices. Therefore, the goal of this work is to demonstrate and to investigate the details of the fusion of viruses with LbL-supported lipid layers. Rubella viruslike particles (RLPs)[13] and Influenza A/PR8 viruses[14] are taken as examples to study and to describe the integration mechanism of these virus nanoparticles into supported membranes. As an add-on, we outline how these composite beads could be used on a chip for diagnostic purposes.

Virus-integrated lipid-coated LbL surfaces consist of three components. A polyelectrolyte multilayer provides the cushion for the SLB. The lipid membrane is then formed on this support by adsorption and spreading of small unilamellar lipid vesicles. The integration of the virus nanoparticles as the biofunctional component is performed by incubating the lipid-coated LbL composites with the viruses at low pH mimicking the conditions within the endosome of mammalian cells. Viruses that have not fused with the lipid membrane will be removed by washing at neutral pH.[15] Although the experiments outlined in this work were exclusively performed with poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS) as polyelectrolyte constituents and as lipids the mixture of phosphatidylserine (PS) and phosphatidylcholine (PC) in a molar ratio of 3:1 was the only one used, further experiments have shown that the protocol can be extended toward other polyelectrolytes as well as other lipids underlining the universality of the approach.

Figure 1 shows atomic force microscopy (AFM) images of RLPs in dry state on the surface of colloidal particles of 20 μm in diameter carrying a LbL-supported lipid membrane. The RLPs were added to the coated colloidal particles and incubated in phosphate citrate (CIP) buffer at pH 4.5 (CIP 4.5), followed by washings in neutral pH buffer (CIP 7.4 or phosphate-buffered saline (PBS)). The RLPs can be identified as spots of about 50 nm in diameter with a height of approximately 20−30 nm. In the corresponding phase image, the RLPs appear as dark spots with a negative phase shift indicating that the RLPs are softer than the noncovered part of the supported lipid layer. The observed height of the particles of 20−30 nm is somewhat reduced compared to its lateral dimensions of the originally spherical particles. This could be a result of the drying process or be due to the lateral force exerted on the hollow viruslike particles resulting

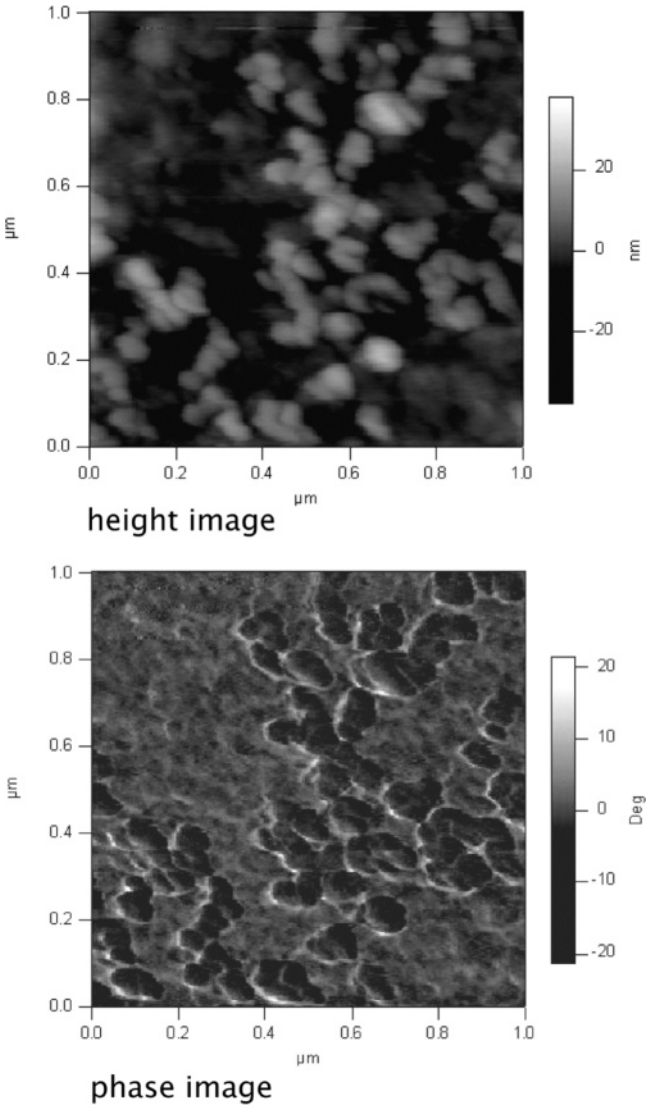

Figure 1. AFM images showing RLPs fused with an SLB taken on the surface of a multilayer-coated colloidal particle in dry state. Top: Height image of the surface with randomly spotted virus nanoparticles. Bottom: Corresponding phase image.

from fusion with the lipid layer. Both reasons may have caused a considerable flattening of the fused viral particles. To resolve the process of RLP adsorption and subsequent fusion in real time, the complete process was followed with a quartz crystal microbalance (QCM) sensor. In brief, QCM sensors were first coated with PAH/PSS multilayers, and subsequently a supported lipid layer either of the same composition as in Figure 1 or with additional cholesterol was assembled. Then the process of RLP adsorption and fusion was followed at two different pH values. The idea was to prove that indeed incubation at low pH values is essential to ensure the firm attachment of the RLPs on the lipid layer, which would be strong evidence for fusion.[16] Figure 2 shows typical results. The QCM frequency shift is plotted versus time. Frequency shifts are a measure for mass changes during the self-assembly processes, and more negative values correspond to an increase of the mass on the sensor. The experiments started with Millipore water flowing constantly over the SLB. Then, the substrates were equilibrated with

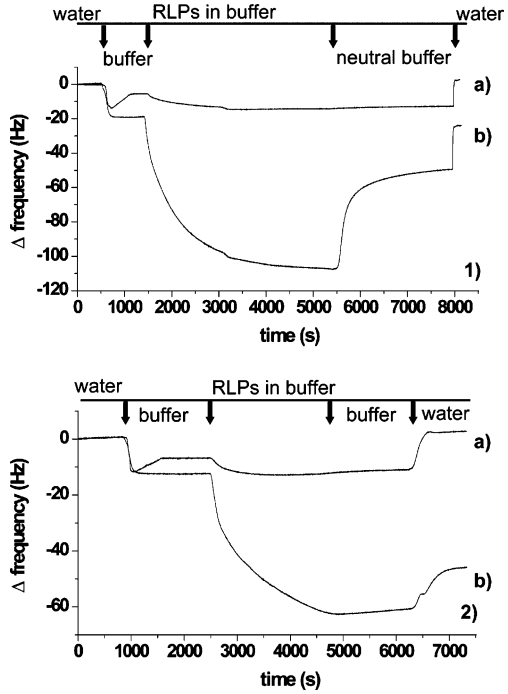

Figure 2. QCM measurements demonstrating the pH-dependence for adsorbing viruslike particles on the SLB. (1, measurement a) RLPs in neutral buffer; (1, measurement b**)** in acidic buffer following washing in neutral buffer and water. (2) A similar experiment with 20% mol cholesterol added to the lipid mixture.

the respective CIP-buffer in constant flow. Next, the RLPs were either applied in constant flow in CIP 7.4 (curve a) or in CIP 4.5 (curve b). It can be seen that at neutral pH values a small amount of the virus particles become adsorbed, which, however, can be completely removed by rinsing with Millipore water at the end of the experiment. The picture is completely different in the acidic buffer. The RLPs in CIP 4.5 adsorb readily at the supported lipid membrane. If this sample is shifted into neutral CIP buffer, a certain proportion of the RLPs desorb from the surface, but the remaining particles are firmly connected to the supported lipid layer as can be concluded from the frequency shift of 25 Hz (Figure 2, panel 1, measurement b) or 45 Hz (Figure 2, panel 2, measurement b) after washing in water. This corresponds to a deposited mass of virus particles on the surface between 6 and 8 mg m^{-2} estimated with the Sauerbrey−Kanazawa approximation implemented in the software of the device. This value is in good agreement with the amount of bound viral protein of 2.5 mg m^{-2} as determined from SDS-PAGE data (see Supporting Information). The difference in the mass determination can be attributed to viral membrane lipids and water within the empty capsid shell. It can be concluded that low pH values both greatly enhance adsorption and likely inititate subsequent fusion of the RLPs with the supported lipid layer but that not all initially adsorbed RLPs proceed toward fusion since a considerable amount can be flushed off with neutral buffer and water. We regard this latter part of the RLPs as nonspecifically adsorbed because the amount of virus particles supplied in the experiment exceeds many times over the number of viruses that can be integrated into the provided area.[11] The nonspecific adsorption occurring in neutral buffer can be decreased by only slightly changing the lipid composition of the SLB, as demonstrated in Figure 2, panel 2, measurement a where 20% (mol mol^{-1}) cholesterol was added to the lipid mixture. Under these conditions, the amount of nonspecifically bound virus particles was reduced by a factor of 2 compared to the lipid mixture without cholesterol employed in Figure 2, panel 1, measurement a. This could be explained by a smaller number of defects in the lipid layer upon addition of cholesterol,[17] assuming that these defects are the sites of nonspecific adsorption, or because of the increased rigidity of the SLB as a result of cholesterol addition that may have reduced nonspecific interactions. The strong interaction of the virus particles with the negatively charged lipid membrane in acidic buffer is likely due to the virus surface becoming positively charged under these conditions. Another parameter that could explain the high affinity of the virus particles toward the SLB at low pH values is the exposure of the hydrophobic fusion peptides of the virus membrane fusion proteins, which is known to be caused by the pH change.

The mechanism of integration of the virus particles into the SLBs can be further resolved employing the lateral diffusion of lipids[18] as a tool to study the interaction of the viral and the supported membrane. The diffusion of lipids was visualized and quantified by means of fluorescence recovery after photobleaching (FRAP) of the SLB. In a first series of experiments, rhodamine, covalently bound to the headgroup of phosphatidyl-ethanolamine (PE-Rho) was used as a marker to study the diffusion in the PS/PC 3:1 lipid mixture. Figure 3a shows images of the lipid-coated colloid taken at the equatorial plane before (1), immediately after (2), and at the end of the experiment (3). The bleached spot (2) is located at the right-hand side of the fluorescent circle representing the coated colloid. The influx of the labeled probe into the bleached area was resolved in time by recording the fluorescence intensity extracted from a stack of images taken at equidistant time intervals during the experiment. Figure 3b shows the fluorescence recovery of the PE-Rho probe in the supported membrane. The experimental data were fitted to the solution of the diffusion equation in spherical coordinates. This yielded a diffusion coefficient of 2×10^{-13} (m^2 s^{-1}). Interestingly, if the lipid-coated LbL colloids were additionally coated with four polyelectrolyte multilayers (Figure 3c) the mobility of the lipid probe was greatly reduced to 3×10^{-15} (m^2 s^{-1}), indicating a pronounced asymmetry in the diffusion coefficients between the inner and the outer leaflet of the supported membrane. The complete and comparatively fast fluorescence recovery of the supported membrane in the absence of additional polyelectrolyte multilayers on top of the lipid layer is likely caused by a combination of fast diffusion in the outer leaflet and a transbilayer movement (flip-flop) of PE-Rho.

Another well-known marker for membrane fusion is octadecyl-rhodamine B (R18).[19] It is a fatty acid derivative with rhodamine bound to its headgroup. It spontaneously incorporates into lipid membranes when added to the bulk. The behavior of this probe is shown in Figure 3d. The

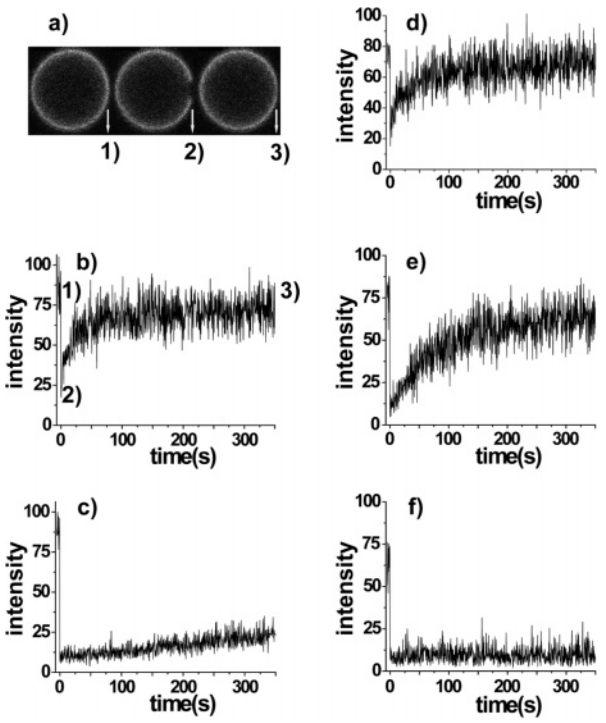

Figure 3. FRAP. (a) Bleaching of a fluorescence-labeled SLB on a LbL colloid of 20 μm in diameter, (1) before, (2) immediately after bleaching, and (3) at the end of the experiment. (b) Fluorescence recovery of PE-Rho in the SLB, no virus. (c) Recovery of the SLB additionally coated with four polyelectrolyte layers, no virus. (d) Fluorescence recovery with the R18 probe inserted in the SLB, no virus. (e) Fluorescence recovery on colloids with fused R18-labeled Influenza A/PR8 viruses. (f) Recovery on colloids with fused Influenza A/PR8 with protein-conjugated rhodamine label.

diffusion coefficient at low concentrations of the R18 label in the PS/PC 3:1 membrane was found to be about 4×10^{-13} ($m^2\ s^{-1}$). If the R18 probe is, however, inserted into the virus particles and the viruses then become fused with the lipid layer on the colloids, the R18 probe will freely diffuse in the resulting membrane indicating mixture of the viral membrane and the SLB. Figure 3e indeed shows a nonrestricted lateral mobility of the R18 probe in the lipid layer of a colloidal sphere into which R18-labeled Influenza A/PR8 viruses have been incorporated. This result provides strong evidence that fusion and subsequent mixing of the two membranes had occurred. Interestingly, the diffusion coefficient of the R18 probe is by a factor of 5 to 10 smaller than in the case when the probe has been directly inserted into the lipid layer, that is, in the absence of incorporated viruses (Figure 3d). There are several possible explanations for this difference. For example, the total area of the membrane increases as a result of the added viral membranes. This in turn would lead to an apparent decrease of the diffusion coefficient. It can also be that the diffusion of the R18 probe is slower because the viral transmembrane proteins may hinder diffusion. The viral envelope contains high concentrations of these proteins. If the Influenza A/PR8 particles were covalently labeled with rhodamine-isothiocyanate on the amino groups of their proteins, a recovery was not observed (Figure 3f), indicating that the immobilized virus particles themselves are firmly inserted into the lipid layer, do not move in the time frame of the bleaching experiment, and hence their capsid is probably in contact with the polyelectrolyte support.

The conventional fusion assay with the R18 label is based on fluorescence dequenching of the R18 probe occurring upon dilution as a result of membrane mixing after fusion. It is often used when studying the fusion of viruses with lipid vesicles as a model system for membrane fusion.[20] Recently, it has also been applied to follow fusion of single viruses with supported lipid layers on glass.[21] While the virus−liposome fusion is usually assessed spectrophotometrically, the fusion of the viruses with the coated colloids can be investigated by means of flow cytometry. Dequenching of the R18 probe on the colloidal surface can be followed in time on the level of single particles. The idea of the experiment is as follows: We take advantage of the nonspecific adsorption of the viruses occurring at neutral pH values, admittedly at a low rate (see Figure 2). Lipid-coated LbL colloids of 3 μm in diameter were incubated with an excess of R18-labeled viruses in neutral PBS buffer. In these experiments, R18 is applied in self-quenching concentrations. At neutral pH, fusion does not yet occur, as demonstrated below. Nonadsorbed viruses in the bulk are then removed by washing. This washing step is important because the subsequent switching of the pH to acidic values would result in additional attachment of viruses from the bulk, rendering the interpretation of the data more difficult. Then, the samples were diluted into either PBS buffer (3), CIP 7.4 (4), or CIP 4.5 (5) and immediately followed in time by flow cytometry. This allows us to quantify the dequenching of the probe fluorescence as a function of time with a resolution of 1 s. Conclusions can be drawn concerning the extent and the course of fusion.

Figure 4a illustrates the major result: that the Influenza A/PR8 viruses begin to fuse with the supported lipid layer immediately after the pH value has been switched to acidic values. The dequenching proceeds albeit with a smaller rate for at least 10 min indicating that more and more adsorbed viruses become fused with the lipid layer after a lag phase that obviously may last for several minutes after acidification of the sample. We would like to rule out the possibility that the individual fusion event of a single virus has dequenching dynamics of the order of 10 min, because the viral dimensions are so small that probe dilution should occur almost instantaneously compared to the time resolution of the flow cytometer once the continuity of the virus and the supported lipid layer has been established by fusion.[21] Colloidal particles with adsorbed viruses remaining in PBS do not show any dequenching. Dilution in CIP 7.4 results in a steady but small increase of fluorescence indicating a nonspecific transfer of the R18 probe at a low rate. Figure 4b shows histograms of Influenza A/PR8 decorated particles before (2) and 10 min after dilution in CIP 4.5 buffer (5). The inset in Figure 4b shows the corresponding dot plots. It can be seen that dequenching of the R18 probe shifts the fluorescence of the entire colloidal population to higher fluorescence values proving the homogeneity of the particle population.

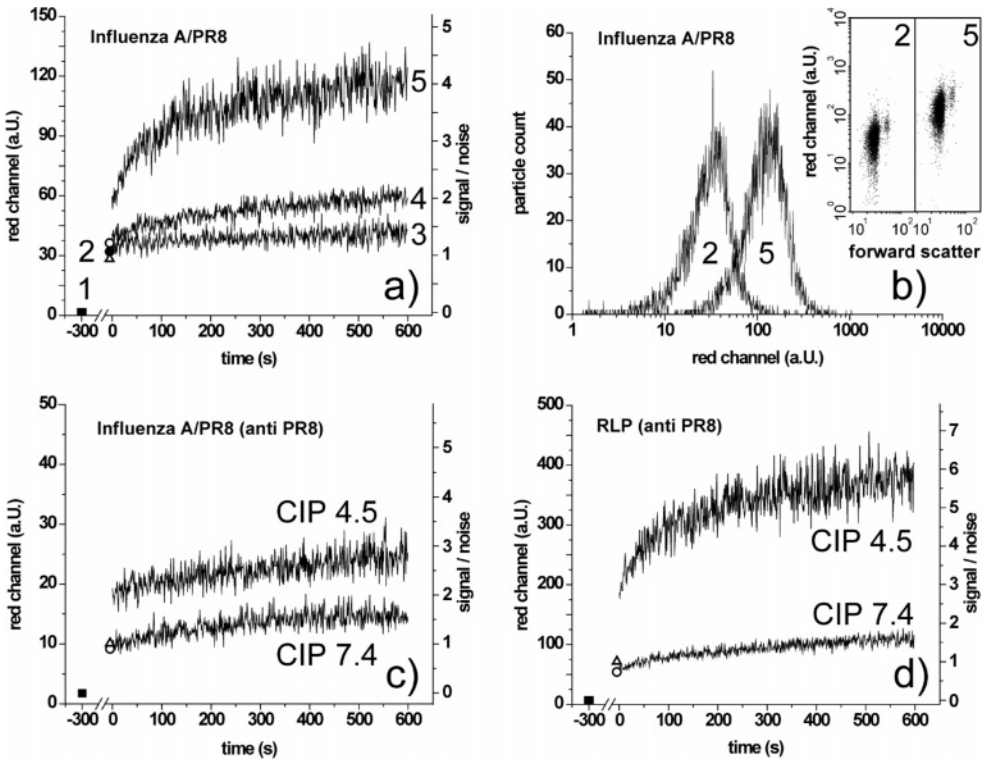

Figure 4. R18 fusion assay with lipid-coated colloids using flow cytometry. (a) Geometric mean fluorescence of: (1) lipid-coated 3 μm LbL colloids without label; (2) composite colloids preincubated in neutral PBS buffer with R18-labeled Influenza A/PR8; (3−5) fluorescence dequenching of colloids diluted with PBS (3), CIP-buffer pH 7.4 **(4),** and pH 4.5 **(**5**)**. Dequenching of the R18 probe in acidic buffer indicates fusion of the adsorbed virus particles with the SLB on the colloids. (b) Histogram of lipid-coated LbL colloids. (2) After incubation with R18-labeled Influenza A/PR8 in PBS buffer. (5) After dilution in CIP buffer, pH = 4.5, for 10 min. Inset: raw data of **(2)** and **(5)**; the small populations at higher scattering representing doublets that were excluded from further analysis by gating. (c) Inhibition of the fusion competence of R18-labeled Influenza A/PR8 by preincubation of the virus particles in polyclonal serum against Influenza A/PR8. (d) RLPs preincubated with the polyclonal serum against Influenza A/PR8. Dilution in CIP, pH = 4.5, results in dequenching of the R18 probe indicating that the polyclonal serum specifically blocks Influenza hemagglutinin but not the fusion competence of the RLPs.

To rule out the possibility that R18 may have been nonspecifically dequenched by exchange rather than by membrane mixing, the Influenza A/PR 8 viruses were preincubated with a polyclonal serum raised in rabbit against the same virus to inhibit fusion.[22] Polyclonal sera contain a variety of antibodies against the accessible epitopes of the virus. Among them there are neutralizing antibodies, which bind to the virus receptor binding epitopes and prevent attachment to the cell, as well as antibodies evoked to the stem domain of influenza virus hemagglutinin containing the fusion peptide. The latter can sterically interfere with the function of the exposed fusion peptide and thus prevent viral fusion. Figure 4c,d demonstrates the specific inhibition of R18-dequenching in the presence of antibodies. The dequenching in acidic CIP 4.5 buffer of the antibody-treated probe was largely inhibited, while the preincubation of the virus particles with polyclonal serum had no effect on the dequenching behavior in neutral buffer, demonstrating that the weak dequenching under these conditions can be likely attributed to nonspecific transfer. Additionally, there was a small initial increase in fluorescence of the particles upon dilution in acidic CIP buffer, which was attributed to the presence of albumin.[23] The albumin was capable of releasing a small amount of R18 from the viral membrane at low pH values. Otherwise, the dequenching characteristics of the R18 probe at low pH in the presence of serum were similar to the behavior of the sample in neutral buffer indicating inactivation of the fusogenic activity of the influenza hemagglutinin by antibodies. This inactivation of virus membrane fusion proteins was virus specific. RLPs preincubated with the polyclonal serum raised against Influenza A/PR8 were not affected by the serum. The dequenching characteristics were the same as in the absence of the antibodies (Figure 4d). Analogous assays were performed with liposomes providing similar results (see Supporting Information).

From the above results, we concluded that the virus particles become at low pH values firmly integrated in the supported membrane. They cannot be removed by washings. This is attributed to fusion of the virus nanoparticles with the lipid-coated LbL composites. The fabricated beads display an authentic viruslike surface. We would like to emphasize the advantages of the presented approach by showing that virus-decorated lipid-coated colloids can be employed as elements in a diagnostic sensor on a chip. We demonstrate the feasibility of this approach for the detection of virus specific antibodies combining microcontact printing[24] with a sandwich technology involving secondary antibodies.

The strong interaction between PAH and the lipid membrane (see Figure 3c) can be used to arrange lipid-coated colloids in defined two-dimensional (2D) structures. A PAH pattern was stamped onto a glass slide. The lipid colloids

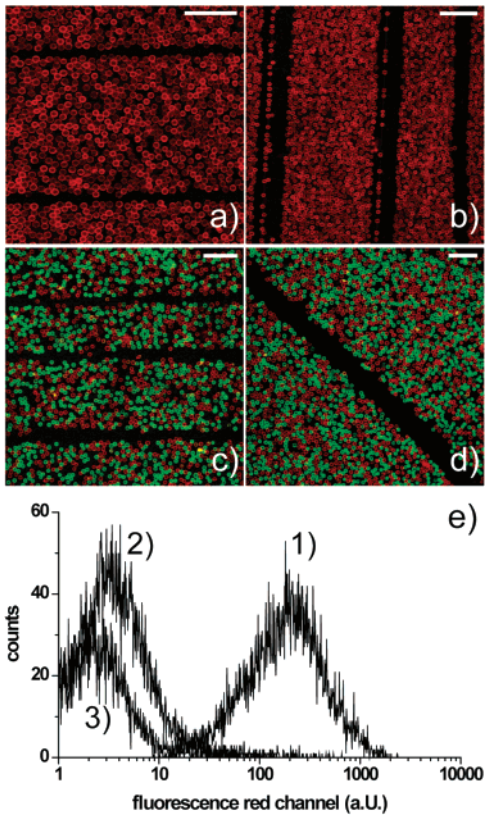

Figure 5. 2D arrangement of composite LbL−lipid−virus colloids on slides patterned with PAH. (a,b) Lipid-coated colloids arranged on a PAH-printed glass slide. (c,d) Immunofluorescence assay on colloids fused with RLPs (green) as negative control and Influenza A/PR8-coated colloids. The red signal indicates specific binding of the primary antibodies as well as the secondary antibody on colloids fused with Influenza A/PR8. Scale bars: 25 μm. (e) Immunofluorescence assay on colloids using flow cytometry. (e, assay 1) Colloids fused with Influenza A/PR8 (geometric mean fluorescence (gmf), 163). Controls: colloids fused with RLPs (e, assay 2, gmf, 3.1), and lipid-coated colloids (e, assay 3, gmf, 2.5). Self-fluorescence of lipid-coated colloids was 2.3 (data not shown).

adsorbed, and the printed pattern was developed by just rinsing the slide with buffer. Colloids adsorbed to the polyelectrolyte pattern stay attached, whereas those from the glass surface were washed away. Figure 5a,b shows patterned red fluorescent lipid-coated colloids. The approach works equally well with virus-decorated colloids, which are then used as sensing elements for detecting virus specific antibodies. Figure 5c,d shows an immunofluorescence assay on patterned colloids carrying either RLPs or Influenza A/PR8. The RLP decorated particles served as the negative control and were now color-coded by incorporation of fluorescent phosphatidyl-ethanolamine-FITC to the lipid layer. The pattern of the two different colloids was incubated in polyclonal sera against Influenza A/PR8. After washing, binding of the influenza specific antibodies to the colloids was detected by using a phycoerythrin-labeled secondary antibody. The secondary antibodies bind to the Influenza A/PR8-coated colloidal sensors with very high specifity as can be seen by the rather small amount of yellow spots. Yellow spots resulting from an overlay of red and green fluorescence correspond to colloids where the secondary antibody was nonspecifically adsorbed to the RLP-coated colloids. Figure 5e shows an analogous immunoassay conducted in solution using flow cytometry. Influenza A/PR8-coated colloids (Figure 5e, assay 1) specifically bind the respective antibodies as evidenced by their large fluorescence (1), while the colloids fused with RLPs (Figure 5e, assay 2) and bare lipid-coated colloids (Figure 5e, assay 3) do not.

We believe that the presented approach of fabricating colloidal particles with selected specific biological properties by means of employing viruses or viruslike particles has a number of advantages. (i) It can be used with a wide variety of viral systems. (ii) Molecular biology techniques developed in the area of virology for various purposes can be used to modify existing and also to design or evolve artificial peptide epitopes on the viruses. Molecular biology and material nanotechnology thus combine into a novel powerful technique. (iii) The lipid layer minimizes nonspecific interactions with biological systems; therefore the system is highly specific and thus especially useful for biomedical applications. (iv) The composite nature of the assembly involves additional degrees of freedom. The LbL layer underneath the lipid as well as the colloidal particle itself may carry a number of additional functions without interfering with virus integration. We thus hope that the presented approach may find applications in science and technology.

Acknowledgment. We thank Christian Elsner (Leibniz Institute of Surface Modification, Leipzig) for PDMS stamps. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) DO 410/4-1 and a grant from University Leipzig (NBL Formel.1-87). S.E.M. is a Ramon y Cajal fellow (Spanish Ministry of Education).

Supporting Information Available: Detailed experimental procedures, SDS-page confirming the presence of virus proteins on virus-coated colloids, R18 fusion assays of viruses with liposomes, and Mathcad-file for calculating diffusion constants. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Fischlechner, M.; Donath, E. *Angew. Chem., Int. Ed.* **2007**, *46*, 3184. (b) Douglas, T.; Young, M. *Science* **2006**, *312*, 873. (c) Singh, P.; Gonzalez, M. J.; Manchester, M. *Drug Dev. Res.* **2006**, *67*, 23. (d) Flynn, C. E.; Lee, S. W.; Peele, B. R.; Belcher, A. M. *Acta Mater.* **2003**, *51*, 5867.

(2) (a) Pelkmans, L.; Helenius, A. *Curr. Opin. Cell Biol.* **2003**, *15*, 414. (b) Klasse, P. J.; Bron, R.; Marsh, M. *Adv. Drug Delivery Rev.* **1998**, *34*, 65. (c) Sieczkarski, S. B.; Whittaker, G. R. *J. Gen. Virol.* **2002**, *83*, 1535.

(3) (a) Weissenhorn, W.; Hinz, A.; Gaudin, Y. *FEBS Lett.* **2007**, *581*, 2150. (b) Dimitrov, D. S. *Nat. Rev. Microbiol.* **2004**, *2*, 109.

(4) (a) Grabherr, R.; Ernst, W.; Oker-Blom, C.; Jones, I. *Trends Biotechnol.* **2001**, *19*, 231. (b) Khare, P. D.; Russell, S. J.; Federspiel, M. J. *Virology* **2003**, *315*, 303.

(5) Noad, R.; Roy, P. *Trends Biotechnol.* **2003**, *11*, 438.

(6) (a) Verhoeyen, E.; Cosset, F.-L. *J. Gene Med.* **2004**, *6*, 583. (b) Lavillette, D.; Russell, S. J.; Cosset, F.-L. *Curr. Opin. Biotechnol.* **2001**, *12*, 461.

(7) (a) Troutier, A.-L.; Ladavière, C. *Adv. Colloid Interface Sci.* **2007**, *133*, 1. (b) Richter, R. P.; Bérat, R.; Brisson, A. R. *Langmuir* **2006**, *22*, 3497. (c) Sackmann, E.; Tanaka, M. *Trends Biotechnol.* **2000**, *18*, 58.

(8) (a) Decher, G. *Science* **1997**, *277*, 1232. (b) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2201. (c) Tang, Z.; Wang, Y.; Podsiadlo, P.; Kotov, N. A. *Adv. Mater*. **2006**, *18*, 3203. (d) Peyratout, C. S.; Dähne, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 3762.
(9) Moya, S.; Donath, E.; Sukhorukov, G. B.; Auch, M.; Bäumler, H.; Lichtenfeld, H.; Möhwald, H. *Macromolecules* **2000**, *33*, 4538.
(10) Schnäckel, A.; Hiller, S.; Reibetanz, U.; Donath, E. *Soft Matter* **2007**, *3*, 200.
(11) Toellner, L.; Fischlechner, M.; Ferko, B.; Grabherr, R. M.; Donath, E. *Clin. Chem.* **2006**, *52*, 1575.
(12) Fischlechner, M.; Toellner, L.; Messner, P.; Grabherr, R.; Donath, E. *Angew. Chem., Int. Ed.* **2006**, *118*, 798.
(13) Qiu, Z.; Ou, D.; Wu, H.; Hobman, T. C.; Gillam, S. *J. Virol*. **1994**, *68*, 4086.
(14) (a) Bentz, J.; Mittal, A. *Biochim. Biophys. Acta* **2003**, *1614*, 24. (b) Nayak, D. P.; Hui, E. K.-W.; Barman, S. *Virus Res.* **2004**, *106*, 147. (c) Skehel, J. J.; Wiley, D. C. *Annu. Rev. Biochem.* **2000**, *69*, 531.
(15) Fischlechner, M.; Zschörnig, O.; Hofmann, J.; Donath, E. *Angew. Chem., Int. Ed.* **2005**, *44*, 2892.
(16) Grutadauria, S.; Cordoba, P.; Cuffini, C.; Zapata, M. *Clin. Diagn. Virol.* **1998**, *10*, 9.
(17) (a) Cannon, B.; Weaver, N.; Pu, Q.; Thiagarajan, V.; Liu, S.; Huang, J.; Vaughn, M. W.; Cheng, K. H. *Langmuir* **2005**, *21*, 9666. (b) Georgieva, R.; Moya, S. E.; Bäumler, H.; Möhwald, H.; Donath, E. *J. Phys. Chem. B* **2005**, *109*, 18025.
(18) (a) Kohli, N.; Vaidya, S.; Ofoli, R. Y.; Worden, R. M.; Lee, I. *J. Colloid Interface Sci.* **2006**, *301*, 461. (b) Kügler, R.; Knoll, W. *Bioelectrochemistry* **2002**, *56*, 175. (c) Cassier, T.; Sinner, A.; Offenhäuser, A.; Möhwald, H. *Colloids Surf., B* **1999**, *15*, 215.
(19) Hoekstra, D.; de Boer, T.; Klappe, K.; Wilschut, J. *Biochemistry* **1984**, *23*, 5675.
(20) (a) Ramalho-Santos, J.; Nir, S.; Düzgünes, N.; Carvalho, A.; Pedroso de Lima, M. *Biochemistry* **1993**, *32*, 2771. (b) Krumbiegel, M.; Herrmann, A.; Blumenthal, R. *Biophys. J.* **1994**, *67*, 2355.
(21) Wessels, L.; Elting, M. W.; Scimeca, D.; Weninger, K. *Biophys. J.* **2007**, *93*, 526.
(22) Edwards, M. J.; Dimmock, N. J. *J. Virol.* **2001**, *75*, 10208.
(23) Donath, E.; Steidel, R. *Acta Biol. Med. Ger.* **1980**, *39*, 207.
(24) (a) Hammond, P. T. *Adv. Mater*. **2004**, *16*, 1271. (b) Nolte, M.; Fery, A. *Langmuir* **2004**, *20*, 2995. (c) Feng, J.; Wang, B.; Gao, C.; Shen, J. *Adv. Mater*. **2004**, *16*, 1940.

NL0723580