VIP Vesicles

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Sweet Talking Double Hydrophilic Block Copolymer Vesicles**

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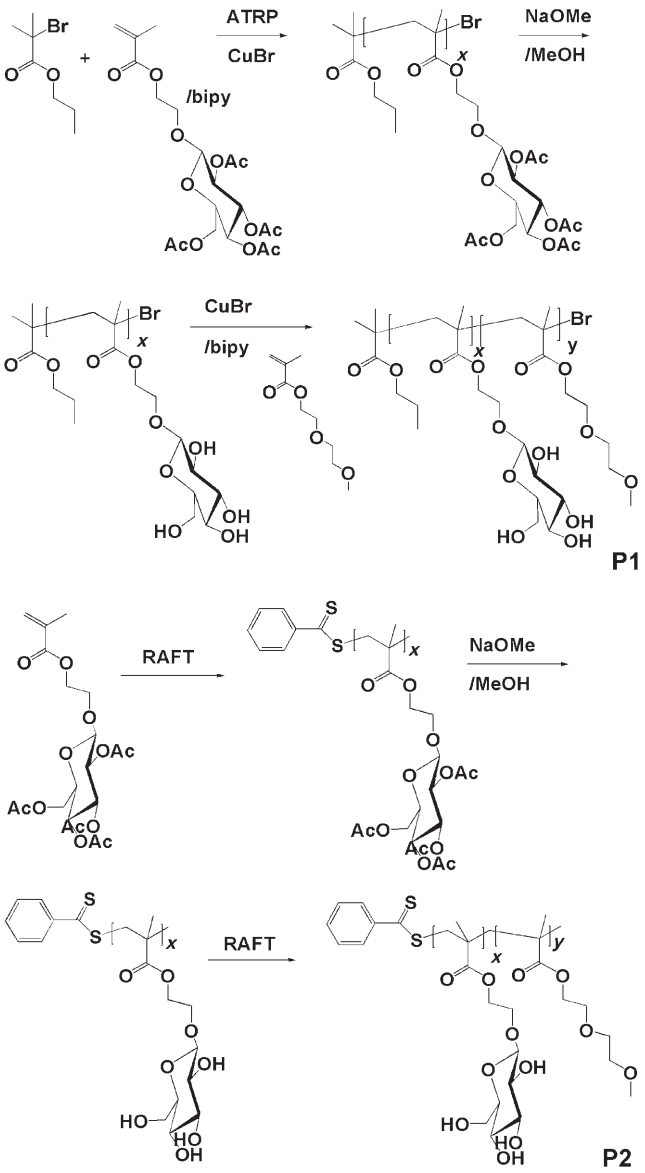
The language of cellular interactions in nature includes a wealth of code based on sugar molecules and macromolecular frameworks.[1,2] The complexity in carbohydrate structure is matched by a subtlety in function that leads to a huge variety of roles for these polymeric sugars in biology.[3,4] It is not surprising therefore that chemists seek to "talk" to biological entities through the language of the glycocode, for this would enable intervention in events such as cell-surface recognition, detection, and signaling, leading in turn to prevention of infectivity,[5] treatment of disease,[6] and even to control of new tissue formation.[7] As our comprehension of the glycocode advances, the use of synthetic sugar-containing polymers to address cells is becoming an area rich with potential.[8–13] Herein we describe an approach towards controlling cell-surface interactions and molecular transport at biointerfaces, by using self-assembling polymer vesicles with multiple copies of a simple glycoligand, glucose, as a first step towards a "conversation" between artificial cell mimics and real cells.[14]

The key to this approach is the preparation of synthetic polymers capable of assembling into capsule-like structures with sugar functionality presented into solution. The aim is to produce a simple mimic of eukaryotic cell surfaces, which might in turn be recognized by biological species that normally bind to glycosylated residues on the cell.[15] To do this, we prepared block copolymers with highly hydrophilic poly(2-glucosyloxyethyl methacrylate) (pGEMA) as one block and more sparingly water-soluble poly(diethyleneglycol methacrylate) (pDEGMA) as the second block by using controlled free-radical techniques (Scheme 1 and Table 1).

We chose glucose as the recognition element, as it exhibits generally weak individual interactions with receptors, but when multiple copies are present on a polymer backbone strong binding can occur through polyvalency.[16] Initial polymer synthesis involved growing a protected precursor polymer[17] by atom transfer radical polymerization (ATRP) or reversible addition–fragmentation chain transfer (RAFT) which was subsequently deprotected to render the block active and hydrophilic. This polymer was used as a macro-initiator to grow the pDEGMA block.

Dynamic light scattering (DLS) showed that below 15 °C, the polymers existed in solution as separate chains, but, at 20 °C, P1 and P2 assembled into vesicles with mean diameters



Scheme 1. Synthesis of glycopolymers P1 and P2.

Table 1: Properties of polymers.

Polymer	M_n [kDa][a]	M_w/M_n[b]	*x*:*y* ratio[b]	LCST [°C][c]
P1	11.2	1.34	10:50	28
P2	15.2	1.11	28:36	28

[a] Measured by gel permeation chromatography. [b] Measured by NMR spectroscopy. [c] LCST = lower critical solution temperature.

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of approximately 251 and 500 nm, respectively. At 37 °C (i.e., at temperatures above the lower critical solution temperature (LCST) of the pDEGMA blocks), the size of the vesicles decreased to around 182 and 300 nm for P1 and P2, respectively, which we attribute to collapse of pDEGMA segments and an increase in the hydrophobicity of the vesicle "cores". Temperature-turbidity assays for P1 and P2 showed broadening and a slight shifting of the LCST onset relative to the pDEGMA homopolymer, which is indicative of a block copolymer structure (Figure 1). The relatively large size of

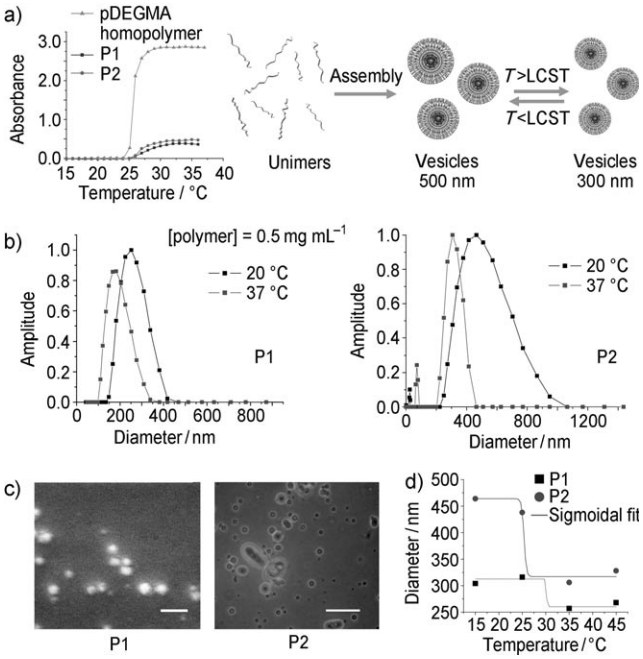

Figure 1. Self-assembly and thermal response of glycopolymer vesicles: a) Temperature-turbidity profiles of P1, P2, and pDEGMA homopolymer; b) DLS data below and above the LCST of the polymer; c) SEM micrographs of P1 and P2 (scale bars 0.5 μm for P1 and 10 μm for P2); d) DLS data on size reduction of vesicles above the LCST of pDEGMA.

some vesicles, particularly those for P2 (ca. 10% of population has greater than 1 μm diameter) conveniently allowed us to examine them by optical microscopy.

Polyvalent binding events at the surface of the vesicles were studied by assays with FITC-concanavalin A, (FITC-con A; FITC = fluorescein isothiocyanate) a lectin with high affinity with glucose that has been extensively used to study carbohydrate-binding interactions.[18] Both P1 and P2 vesicles were able to accommodate the lectin at their surfaces, and turbidity assays showed that vesicles could agglutinate con A more efficiently than a model GEMA homopolymer (ESI). Agglutination with con A was more pronounced in the case of smaller vesicles made by P1 than P2 (corresponding to around 30 and 60% increased con A absorbance for binding to P2 and P1, respectively). The vesicular structures seemed to provide two major advantages over linear homopolymer systems: 1) spatial accumulation of sugar moieties on the vesicular corona provided increased multivalent capacity and 2) variations of vesicular size resulted in changes in the overall association of biomolecule–vesicle complexes/aggregates.

We next studied the binding activity of the vesicles with a mutant *E. coli* strain (MG1655pGFP) that is both fluorescent (GFP) and expresses the *fim*H protein, which has binding specificity for glucose and mannose. *E. coli* are rod-shaped bacteria of comparable size to the larger P2 vesicles, and their binding characteristics with linear glycopolymers have been studied previously.[19,20] The carbohydrate recognition sites (CRSs) found on the pili of *E. coli* are a few nanometers in diameter and can reach more than 3 μm in length.

Figure 2 shows the varying interactions of the different vesicles with *E. coli*. The small vesicles from P1 formed large aggregates with bacteria (40–80 μm^2, approximately 100–

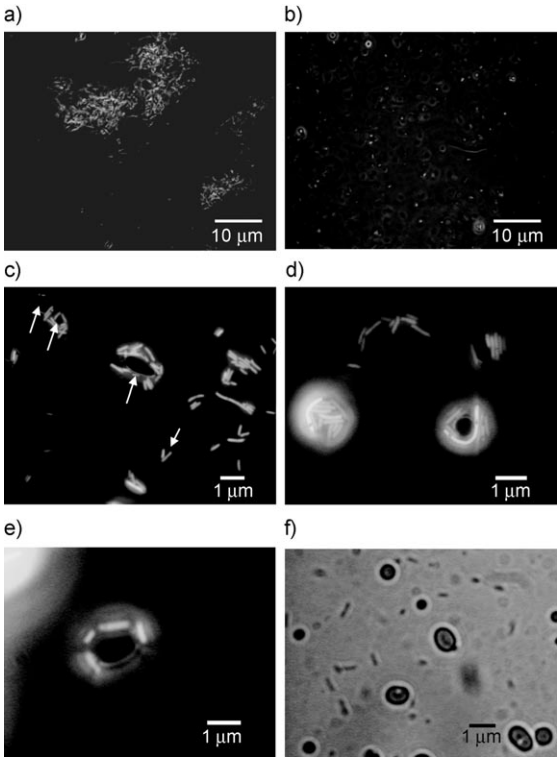

Figure 2. Association of vesicles with bacteria: a) large aggregates of *E. coli* MG1655pGFP (*fim*H positive) with P1; b) P1 with *E. coli* Top 10 (*fim*H negative). Large (>1 μm) P2 vesicles bind but do not aggregate with *E. coli* MG1655pGFP as shown in images (c–e) in fluorescence mode; f) no binding of *E. coli* Top 10 to P2 vesicles is observed in phase-contrast mode.

150 bacteria and 60–90 vesicles found in each cluster). In contrast, for P2, no large-area aggregates were formed, although we did observe persistent strong individual bacteria associations with large (ca. 1 μm vesicles) in the mixture (see movie in the Supporting Information). Negligible bacterial aggregation was induced when the vesicles interacted with an *E. coli* strain (Top 10) that did not express *fim*H, thus demonstrating the specific nature of the binding process owing to the sugar functionality. The induction of bacterial cluster formation followed the same trend as with con A

interactions, which we attributed to the differing size, mass, surface-volume ratios, and momentum in suspension of P1 relative to P2 vesicles (see the Supporting Information).

Having established that polymer-vesicle binding involved surface-expressed glucose as the "language" of cell–vesicle interactions, we sought to "outtalk" the association through introduction of exogenous signals (i.e., free glucose). Addition of glucose into preformed bacterial-vesicle aggregates resulted in dose-dependent breakdown of the cell–polymer clusters (Figure 3a–d). This effect was most noticeable for the smaller vesicles from P1, but was also apparent in the mixture of P2 vesicles with *E. coli*.

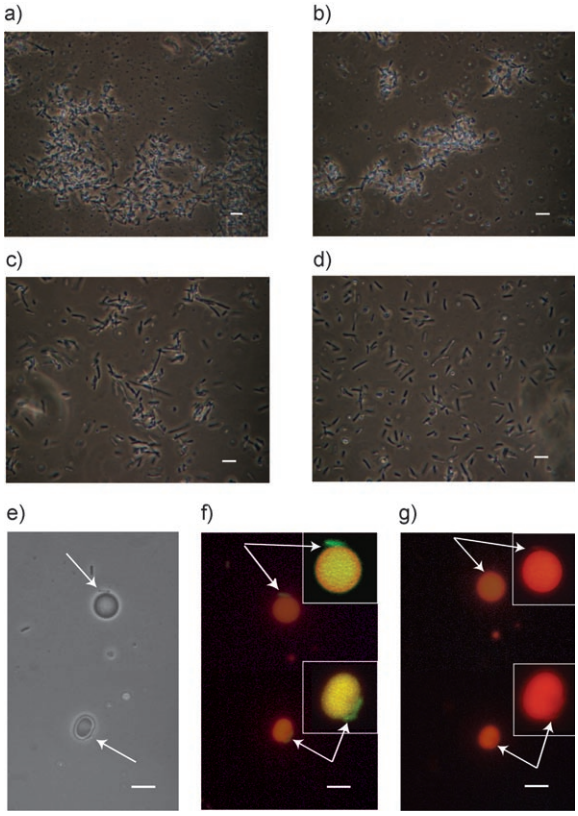

Figure 3. Information transfer at vesicle-cell interfaces: a–d) P1–*E. coli* aggregates before (a) and after addition of 0.05 (b), 0.5 (c), and 5 mM glucose (d). e–f) Molecular transport from P2 vesicles to *E. coli*. Image (e) shows vesicles and cells in phase-contrast mode, (f) shows the same cells in fluorescence mode; bacteria fluoresce green (GFP) and vesicles containing ethidium bromide fluoresce orange-red. Image (g) shows the same vesicle–cell partners after 30 min with bacteria now fluorescing orange-red owing to transfer of ethidium bromide. Insets in (f) and (g) show vesicles at higher image contrast and ×2 magnification for clarity. Scale bars 1 μm.

It was more difficult to quantify the binding interactions of P2 and *E. coli* and the breakup of aggregates/clusters owing to the increased random motion in solution of the larger vesicles (see movie in the Supporting Information). However, for P2, polymers binding events of single vesicles with individual bacteria could be observed, which we reasoned might allow us to investigate a second mode of "communication" between vesicle and cell, namely, molecular transport. Our hypothesis was that the interfacial interaction of vesicles with bacteria might trigger disruption of the vesicular membrane and therefore vesicles containing molecular "information" could communicate this "information" when in contact with the bacteria. Large vesicles from P2 were loaded with the dye ethidium bromide and cell–vesicle interaction studies were performed. As is apparent from Figure 3e–g, bacteria associated with vesicle surfaces were initially green through GFP fluorescence, but over time (30 min) fluoresced orange-red through transfer of ethidium bromide from the vesicle interior to the bacterial cytoplasm. Only cells attached to vesicles exhibited ethidium bromide uptake over this time period, thus establishing the specificity of the information transfer.

These data demonstrate that not only can specific interactions between synthetic vesicles and cells occur, but also that a degree of control can be exerted in the information conveyed in these interactions. By changing the vesicular size it is possible to change an interaction from bulk aggregation to individual associations. This in turn might lead to a sensitive method of cell detection or for control of signaling to individual cells. Other recent studies have correlated binding capabilities of molecular glycoobjects[21] to their supramolecular architecture, with specific goals of bacterial detection.[22–24] Our results imply that not only might the affinity of these materials be optimized by noncovalently linked supramolecular assemblies, but also that perhaps information transfer between cells and vesicles[25–27] might be achieved by rational design.

In conclusion, we have designed new block copolymers that assemble into vesicles with surface display of glucose functionality. The vesicle sizes can be controlled by comonomer content, block ratio, molar mass, and LCST.[28] This control allows information transfer to biological cells either through the glycosylated surface, or through the contents of the vesicle interior. The vesicles can thus be considered as a mimic, albeit primitive, of natural cells with their associated glycocalyx, with potential applications in cell sensing, therapeutics, and synthetic biology.

Experimental Section

Synthesis of polymer P1: Acetylated glucosyloxyethyl methacrylate (AcGEMA) was synthesized according to a published procedure.[17] In an N_2-purged round-bottom flask, AcGEMA (3 g, 6.5 mmol) was dissolved in degassed MeOH (8 mL). The flask was sealed with a rubber septum and a degassed solution of CuBr (123 mg, 0.8 mmol) and 2,2-bipyridyl (bipy, 269 mg, 1.7 mmol) in MeOH (4 mL) was added through a glass syringe. Then, the initiator ethyl-2-bromoisobutyrate (128 μL) was added through a microsyringe and the flask was placed in an oil bath at 30 °C. After 90 min, the polymerization was stopped by exposing the reaction mixture to air. The resulting poly(AcGEMA) was isolated by dialysis against acetone using a cellulose 1000 molecular-weight cutoff (MWCO) membrane. Deprotection of poly(AcGEMA) involved dissolving the polymer in a mixture of $CHCl_3$/MeOH (1:1) (0.5 g mL^{-1}). A catalytic amount of NaOMe in MeOH was added to the solution (1 mM). The reaction was left under an atmosphere of N_2 until the solution became turbid. Poly(GEMA) was isolated by centrifugation and lyophilization. Poly(GEMA) was used as macroinitiator to grow the pDEGMA block as follows: Diethylene glycol methacrylate (890 mg, 4.7 mmol)

was added in a previously N_2-purged round-bottom flask. The flask was sealed with a rubber septum and a freshly prepared solution of CuBr (13.6 mg, 0.1 mmol), bipy (29.8 mg, 0.2 mmol), and poly-(GEMA, 200 mg) in MeOH (4 mL) was added through a glass syringe. The flask was placed in an oil bath at 60 °C for 3 h. The reaction was stopped by exposing the reaction solution to air. The final product was recovered by dialyzed against water for 3 days and then freeze drying.

Synthesis of polymer P2: AcGEMA (5 g, 10.8 mmol) was dissolved in MeOH (5 mL) in a 50 mL round-bottom flask. Azobis-isobutyronitrile (AIBN, 7.5 mg) and RAFT agent cyan-2-yldithio-benzoate (CDBA, 47.5 mg) were added to the flask, which was then purged with N_2 for 20 min. The rubber-septum-sealed flask was placed in an oil bath at 70 °C overnight, and then the reaction was stopped by opening the flask to air. The precursor polymer was recovered by dialyzing the reaction mixture against excess MeOH using 1000 MWCO membrane for 2 days. Deprotection was carried out as described for P1. The pDEGMA block was grown as follows: pGEMA (1 g) and DEGMA (1 g) were dissolved in EtOH (3 mL). AIBN (2.8 mg) and CDBA (19 mg) were added. The flask was sealed and the reaction mixture was purged with N_2 for 20 min, and the reaction was allowed to proceed overnight at 65 °C. The polymer was recovered by dialysis against water for 2 days and subsequent freeze drying for 24 h.

Bacteria–vesicles interactions: Bacteria were grown in lysogeny broth (LB medium) up to the stationary phase (optical density = 0.8) by incubation overnight at 37 °C in the dark. Aliquots (300 μL) were collected in eppendorf tubes and centrifuged at 13000 rpm for 3 min. The supernatant was then replaced with freshly prepared phosphate-buffered saline (PBS; pH 7.4) containing the polymer (4 mg mL^{-1}). The samples were vortexed for 5 s and left to settle for 15 min at room temperature(ca. 25 °C). Aliquots (10 μL) of the sample were collected with a micropipette, mounted on a glass slide with a cover slip on top, and examined with an optical microscope. Control experiments were also conducted using a precursor polymer (see the Supporting Information).

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[28] Estimates of sugar content based on an approximate glycoblock head-group area of 15 nm^2 (Chem 3D software) suggest that there were approximately 2.4×10^4 glycopolymer blocks at the surface of each P1 vesicle, whereas for 1 μm P2 vesicles there were probably nearer to 7.5×10^5 glycoblocks. The higher block length of the glycopolymer segment and thus its greater hydrophilicity in P2 relative to P1 was the most likely reason for the changed size and curvature of the P2 vesicles relative to P1.

