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Polymer Vesicles with a Colloidal Armor of Nanoparticles

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Supporting Information

ABSTRACT: The fabrication of polymer vesicles with a colloidal armor made from a variety of nanoparticles is demonstrated. In addition, it is shown that the armored supracolloidal structure can be postmodified through film-formation of soft polymer latex particles on the surface of the polymersome, hereby effectively wrapping the polymersome in a plastic bag, as well as through formation of a hydrogel by disintegrating an assembled polymer latex made from poly(ethyl acrylate-co-methacrylic acid) upon increasing the pH. Furthermore, ordering and packing patterns are briefly addressed with the aid of Monte Carlo simulations, including patterns observed when polymersomes are exposed to a binary mixture of colloids of different size.

Amphiphilic molecules placed in a liquid medium can self-assemble into a variety of suprastructures. One type is unilamellar vesicles, hollow bilayer-based membrane sacs that contain fluid. The availability of a plethora of synthetic macromolecular amphiphiles through advances in living polymerization methods has led to a surge in the preparation of vesicles made from polymer molecules, coined polymersomes.[1,2] Polymer vesicles have interesting chemical and physical properties, which outperform synthetic liposomes made from phospholipids. One of the key features is that these polymer nanocontainers are more mechanically robust, as a result of their increased bilayer thickness,[3] which makes these hollow structures interesting as drug delivery vehicles.[4] Tailored synthesis of the macromolecular building blocks provides added complexity and functionality to their design. Use of biodegradable,[5] oxidative responsive,[6] or pH/sugar responsive blockcopolymers[7] in the fabrication of polymer vesicles allows for triggered bilayer disintegration inducing permeability or vesicle rupture. Examples focusing on mechanical reinforcement include the ability to cross-link the bilayer of polymer vesicles made from poly(ethylene oxide)-*block*-polybutadiene,[8,9] or provide a polymeric scaffold through intrabilayer polymerization.[10]

Our idea is to add functionality and potentially enhance mechanical strength to polymersomes by decorating their outer surface with an armor of colloidal matter. We took our inspiration from Nature, how it safeguards mechanical strength in certain classes of cells and organisms. In addition to the mechanical strength provided by the cytoskeleton of the cell, plants, fungi, and certain bacteria have an additional cell wall as outermost boundary. Organisms that attracted our interest were ones with a cell wall composed of an armor of colloidal objects, for example, bacteria coated with S-layer proteins,[11] and coccolithophorids which have a $CaCO_3$-based nanopatterned colloidal armor.[12] Velev demonstrated that synthetic liposomes could be coated with a layer of ferritin.[13] Weitz and co-workers showed that crystalline rafts of microspheres could be formed on the outside of vesicles made from mixed low-molar-mass surfactants.[14,15] Noteworthy is the work by Lecommandoux and co-workers who prepared polymer vesicles which had magnetic maghemite nanoparticles incorporated into the hydrophobic region of the bilayer.[16] We use electrostatic attraction as drive for assembly on the outside of the bilayer. Caruso and others have shown by using a layer-by-layer approach that electrostatic attraction can be used successfully in the preparation of a great variety of nanoparticle hybrid capsules.[17] Wooley and co-workers decorated cylindrical micelles with shell-cross-linked knedel-like nanospheres.[18] In our work we show not only that we can provide polymer vesicles with a colloidal armor made from a variety of nanoparticles, but also that we can postmodify the supracolloidal structure through film-formation and formation of a hydrogel. Furthermore, we will briefly address ordering and packing patterns, including patterns observed when polymersomes are exposed to a binary mixture of colloids of different size.

We prepared polymer vesicles from poly(*n*-butyl methacrylate)-*b*-(*N*,*N*-dimethylaminoethyl methacrylate) block copolymers. This block copolymer was made via atom transfer radical polymerization (see Supporting Information). Unilamellar polymersomes were formed by slow addition of an excess amount of water at pH 5 (90 mL) at a rate of 1.5 mL min^{-1} to a 10 mL solution of the block copolymer in tetrahydrofuran (THF), at 1.0 g L^{-1}.

The THF was removed by dialysis against water of pH 5. This was to warrant protonation of the *tert*-amino groups (pK_a, 8.5). The unilamellar nature of the cationic polymersomes was confirmed by cryogenic transmission electron microscopy (cryo-TEM). Dynamic light scattering measurements showed an average diameter of approximately 1.0 μm with a dispersity of 0.11 indicating a broad size distribution of vesicles (see Supporting Information)

We made use of electrostatic attraction of negatively charged colloids onto our positively charged polymer vesicles as adhesion force. Assembly took place through collision of the colloidal particles with the polymersome, hereby relying on Brownian motion. Typically, to a 2 mL polymersome dispersion in water at pH 5, 0.1 g of a 1 wt % aqueous dispersion of colloids was added. Figure 1a is a cryogenic scanning electron microscopic (cryo-SEM) image of a collection of polymersomes armored with a layer of monodisperse polystyrene latex particles (average particle diameter ca. 190 nm, for additional images see Supporting Information). Note that no adhesion of the anionic polystyrene

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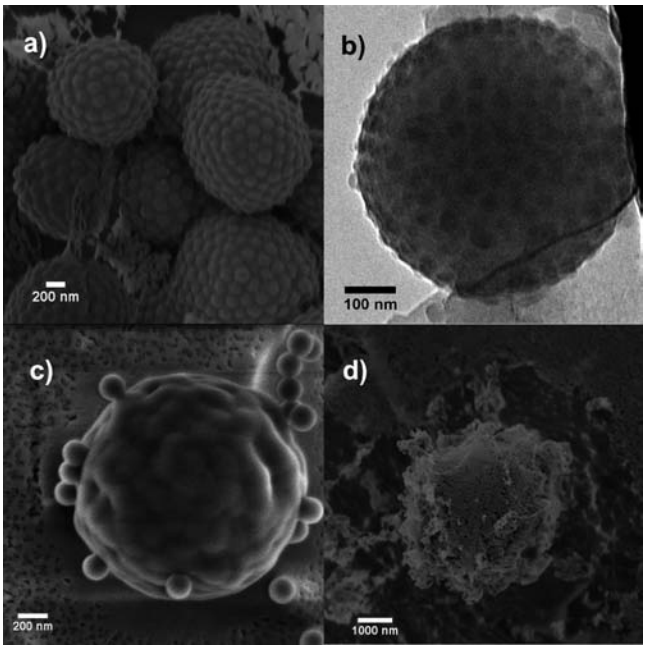

Figure 1. Cryo electron microscopy images of polymer vesicles armored with (a) polystyrene latex spheres, (b) silica nanoparticles (Ludox TM-40), (c) partially film-formed poly(*n*-butyl methacrylate) latex particles, and (d) a poly((ethyl acrylate)-*co*-(methacrylic acid)) hydrogel originated from pH responsive polymer colloids.

particles was observed when polymersomes made from poly(*n*-butyl methacrylate)-*b*-(polyethylene oxide) were used as template. Images resemble the assembly of particles on emulsion droplets, thereby protecting the droplet from coalescence through Pickering stabilization.[19−21] What is stricking in this image is the packing order of the polystyrene spheres onto the surface of the vesicle. Collisions as a result of Brownian motion are random, which implies that particles can rearrange themselves once adhered, and/or that adhesion is reversible. This ordering process occurs in order to achieve the optimal packing configuration through minimization in free energy. The use of monodisperse particles plays a key role in achieving the high packing order and thus 2D crystallization of the particles on the soft interface.[22,23] Employment of particles with a broader size distribution would reduce order fading out grain boundary scars.[23] The time scale to fully cover the polymersomes can be estimated by linking packing patterns to the Smoluchowski and Stokes−Einstein diffusional equations, with indicative values in the order of a minute.[21] To relax the system and obtain ordered packing patterns, an annealing time exceeding this time scale by several orders of magnitude needs to be allowed. Indeed a more random arrangement of polystyrene spheres onto the polymer vesicles would be observed if the samples were quenched and analyzed by cryo-SEM after 10 min of incubation time. In our annealed systems, we did not observe any Fibonacci number patterns, which suggest that the armored polymersomes can relax to adopt a stress-free packing geometry.[24]

We were interested in exploring the versatility of our method. We therefore not only investigated polystyrene latex particles as colloidal building blocks for our supracolloidal armor, but also used silica nanoparticles, "soft" polymer latex spheres, and pH-responsive latex spheres. Cryo-TEM analysis (see Figure 1b, and Supporting Information) shows that indeed our cationic

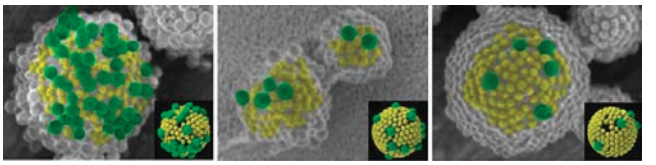

Figure 2. Micronsized polymersomes armored with a mixture of large (200 nm) and small (120 nm) polystyrene latex particles. Encounter probabilities of small beads are 56%, 78%, and 91%, respectively. Insets show corresponding MC simulated packing patterns.

polymersomes can be armored with a layer of silica nanoparticles of average diameter of approximately 24 nm (Ludox-TM40), resulting in a hybrid organic−inorganic vesicular structure. It is evident from this image that the hollow bilayer-based structure of the vesicle is preserved and that the particles are adhered to the outer surface. Packing patterns of the silica nanoparticles are less ordered as a result of their more polydisperse nature.

We asked ourselves the question whether we could induce autohesion and thus film-formation of an armor of "soft" polymer latex spheres, effectively wrapping the polymer vesicle in a plastic bag. We therefore used negatively charged poly(butyl methacrylate) latex particles as colloidal building blocks. From the cryo-SEM analysis (Figure 1c), it can indeed be observed that the armor no longer is composed of a collection of discrete assembled polymer latex spheres, but that the particles have undergone partial film formation. We feel that this process can not only be of great value to control the overall rigidity of the reinforced polymersomes, but also can be an effective tool to alter its permeability and thus control for the release or uptake of drugs.

We also provided our polymersomes with a supracolloidal armor of latex spheres which had the ability to dissolve partially and form an aqueous based gel-phase. We hereby make use of a waterborne polyHASE, which consists of polymer latex spheres made from a mixture of ethyl acrylate (60 wt %) and methacrylic acid (40 wt %). Emulsion polymerization at low pH yields latex spheres which upon pH increase to approximately 7.0 unwrap their polymer chains and expand into a gel. The result is a polymersome with a stealth layer of aqueous gel, as clearly can be observed in Figure 1d.

Finally, we briefly explored with the aid of Metropolis Monte Carlo (MC) simulations (see Supporting Information for details) which packing geometries of supracolloidal armor would form after a minimum annealing time of 14 h if we exposed our polymer vesicles (approximately 1 μm) to a binary mixture of anionically charged polystyrene latex spheres of different size, 120 and 200 nm in diameter, respectively. The polymer latex particles are modeled as semi soft spheres interacting via a narrow 12−24 Lennard−Jones (LJ) potential. Electrostatic contributions were taken into account using a varying Yukawa potential. The MC simulations are corrected for a different collision flux of the two sets of latex particles as a direct result of their difference in size and thus their variation in Brownian velocity (see Supporting Information.).

The ratios of beads observed in the electron micrographs most closely resemble the simulations with a medium strength Yukawa potential of A = 10 000, implying Coulombic interactions play a role showing a long-range repulsion between the large particles, and to a lesser extent between the large and small ones. The interactions between the small particles are dominated by the Lennard−Jones potential (Figure 2). In effect the large particles distort the organized packing of the small ones.

In summary, we have demonstrated that polymer vesicles can be decorated with an armor of nanoparticles. We believe that this approach opens interesting pathways in the already versatile application areas of polymersomes.

■ ASSOCIATED CONTENT

Supporting Information. Details on experimental conditions and characterization of the block copolymer, polymer latexes and armored polymersomes. Dynamic light scattering data for the bare polymersomes. Computational details for the Monte Carlo simulations. Additional Electron Microscopy images. This material is available free of charge via the Internet at http://pubs.acs.org.

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