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Layer-by-layer assembly of polymersomes and polyelectrolytes on planar surfaces and microsized colloidal particles

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ABSTRACT

Hybrid polyelectrolyte multilayer systems were fabricated on top of planar surfaces and colloidal particles via layer by layer (LbL) assembly of polystyrene sulphonate (PSS) and polybenzyl methacrylate-*block*-poly(dimethylamino)ethyl methacrylate (PBzMA-*b*-PDMAEMA) polymersomes. Polymersomes were prepared by self assembly of PBzMA-*b*-PDMAEMA copolymer, synthesised by group transfer polymerisation. Polymersomes display a diameter of 270 nm and a shell thickness of 11 nm. Assembly on planar surfaces was followed by means of the Quartz Crystal Microbalance with Dissipation (QCM-D) and Atomic Force Microscopy (AFM). Detailed information on the assembly mechanism and surface topology of the polymersome/polyelectrolyte films was thereby obtained. The assembly of polymersomes and PSS on top of silica particles of 500 nm in diameter was confirmed by ζ-potential measurements. Confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) revealed that polymersome/PSS coated silica particles increase in total diameter up to 3–5 μm. This hints toward the formation of densely packed polymersome layers. In addition, CLSM showed that polymersome/PSS films exhibit a high loading capacity that could potentially be used for encapsulation and delivery of diverse chemical species. These results provide an insight into the formation of multilayered films with compartmentalised hydrophilic/hydrophobic domains and may lead to the successful application of polymersomes in surface-engineered colloidal systems.

1. Introduction

The tunable construction of thin-film-based nanoarchitectures via layer-by-layer (LbL) assembly has opened up new horizons in materials science and led to exciting new developments in many scientific areas during the past two decades [1–4]. One major advantage of LbL assembly is the intrinsic potential for the combination of diverse building blocks through complementary interactions, i.e.: electrostatic interactions, hydrogen bonding, etc., to create thin films displaying functional groups and chemical entities at controlled sites in nanoscale arrangements. From the standpoint of methodology, the LbL toolbox [5] is currently able to offer a broad spectrum of nanocomposite thin films [6] compatible with multiple applications i.e.: drug delivery, antifouling coatings, nanodevices, membranes for nanofiltration, etc. [7–12].

Initially, LbL assembly was developed to produce nanoscale films through the alternate deposition of oppositely charged polyelectrolytes [13]. Thereafter, the LbL method was successfully extended to other building blocks. A broad variety of multilayer composite films have been constructed by replacing one or both polyelectrolyte counterparts with other charged building blocks such as proteins [14], dendrimers [15], lipids [16] and colloidal nanoparticles [17]. In this regard, LbL assemblies fabricated upon micelles [18,19] or vesicles [20] have proven to be very efficient in confining and hosting different chemical species within the LbL film. The use of "soft" nanocapsules to promote the selective loading of predefined host molecules into LbL thin films represents a research topic of growing relevance in supramolecular materials science [21,22]. For example, Zhang and co-workers have proposed the use of block copolymer micelles as matrices for the

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incorporation of organic species and the fabrication of LbL films by the alternate deposition of the block copolymer micelles and polyelectrolytes [23].

In a similar vein the integration of lipid vesicles [24] into polyelectrolyte multilayers has also shown great versatility to enhance the loading capacity of the multilayers for potential drug delivery applications. However, lipid vesicles have very poor stability and easily rupture when interacting with polyelectrolytes during LbL assembly [25]. Within this framework we should emphasise that the incorporation of polymer vesicles in LbL assemblies remains almost completely unexplored [26]. Polymeric vesicles, also known as "polymersomes", are hollow, lamellar spherical structures (self-assembled polymer shells) composed of block copolymer amphiphiles [27,28]. Hydrophobic blocks of each copolymer aggregate and minimise direct exposure to water whilst hydrophilic blocks form inner and outer hydrophilic layers in contact with aqueous media, thus resembling a typical bilayer vesicle formed by lipids [29]. However, compared with lipids, the larger molecular weight and restricted conformational freedom of block copolymer polymer chains endow polymersomes with enhanced durability and reduced water permeability [30]. In recent years polymersomes have received considerable attention within the materials science community due to their improved mechanical properties, high stability, and ability to incorporate compounds not only in the aqueous core but also in the hydrophobic domains [31,32].

In this context, polymersomes may result in interesting building blocks to create LbL assemblies exhibiting high loading capabilities. Such assemblies could be applied for oil, perfume, dye encapsulation in surface coatings as well as for drug delivery, especially in topical applications where there may be safety concerns related to the chemistry of the polymers. A thorough understanding of multilayer formation with polymersomes is essential for exploring new classes of materials to engineer LbL assemblies. Therefore, this work aims at providing a deeper insight into the self assembly of polymersomes with polyelectrolytes in LbL films. As building blocks for the films we have chosen polymersomes fabricated upon polybenzyl methacrylate-*block*-poly(dimethylamino)ethyl methacrylate block copolymer, which is positively charged, and polystyrene sulphonate, a negatively charged polyelectrolyte. Since polymersomes are themselves self assembled particles the films fabricated on the basis of alternating polymersomes and polyelectrolyte deposition will have a higher degree of organisation than standard LbL films.

We first explored the mechanism of assembly of polymersomes with the Quartz Crystal Microbalance with Dissipation (QCM-D) and Atomic Force Microscopy (AFM). Polymersomes are quite large structures in comparison with the counter polyelectrolytes. Because of this feature we observed that the coating of planar surfaces by LbL assembly of polymersomes and polyelectrolytes resulted in a discontinuous arrangement of polymersomes. However, a completely different situation was observed when the LbL process is performed on top of colloidal particles, where the polymersomes form large shells around the support colloidal particle after 4 assembly steps. We observed that the assemblies are indeed significantly larger than the particles on top of which of the coating was grown. The difference in the structures produced by the assembly of polymersomes on top of planar and spherical surfaces are explained on the basis of the differences in the assembly protocols. In particular, the use of centrifugation during the coating of the colloidal particles generates a high concentration of polymersomes around the silica particles that are responsible for the formation of the thick polymer shell. The large polymersome assemblies produced on top of silica particles could have applications in encapsulation and drug delivery. ζ potential measurements, Confocal Laser Scanning Microscopy (CLSM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to characterise the LbL assembly on colloidal particles.

2. Experimental

2.1. Chemicals and reagents

Poly(allylamine hydrochloride) (PAH) ($M_w \sim 15{,}000$), poly(sodium 4-styrenesulphonate) (PSS) ($M_w \sim 70{,}000$), benzyl methacrylate (BzMA, 96%), 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%) monomers, the initiator, 1-methoxy-1-trimethylsiloxy-2-methyl propene (MTS), tetrabutylammonium hydroxide and benzoic acid, polystyrene sulphonate (PSS) (M_w 70,000), NaCl, rhodamine B were all purchased from Sigma–Aldrich. Monomers were purified by vacuum distillation and stored in the presence of activated 4A molecular sieves before use. Chloroform (Carlo Erba, 99%) for HPLC was used as purchased. Tetrahydrofuran (THF, Carlo Erba, 99.8%), the polymerisation solvent, was refluxed under nitrogen over sodium (Na)/benzophenone (BP) in a controlled atmosphere still until blue colour of medium. The THF was then distilled into a flask with activated 4A molecular sieves and stopped with septum stopcock. The polymerisation catalyst, tetrabutylammonium bibenzoate (TBABB), was prepared from the reaction of tetrabutylammonium hydroxide with benzoic acid according to Dicker et al. [33] and was kept under vacuum until use.

2.2. Block copolymer synthesis and characterisation

Diblock copolymer of benzyl methacrylate (BzMA) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) units, was prepared by group transfer polymerization [34,35] (GTP) in THF using glass reactor under dry nitrogen and standard Schlenk techniques as described elsewhere [36]. The chemical structure was determined by ^{1}H NMR employing a Varian-200 MHz (Mercury 200) at 35 °C in $CDCl_3$. Tetramethylsilane (TMS) was used as an internal standard. δ(ppm): 1.06–0.73 ($-C\mathbf{H}_3$, m, 6H); 1.91–1.79 ($-C\mathbf{H}_2-$, m, 4H); 2.28 ($-N(C\mathbf{H}_3)_2$, s, 6H); 2.56 ($-C\mathbf{H}_2-N<$, t, 2H); 4.06 ($-O-C\mathbf{H}_2-$, t, 2H); 4.90 ($Ar-C\mathbf{H}_2-$, s, 2H); 7.28 ($Ar\mathbf{H}$, m, 5H). The molecular weight distribution and average molecular weights were determined by size exclusion chromatography (SEC), using a LKB-2249 instrument at 25 °C. A series of four μ-Styragel® columns with pore sizes of 10^5, 10^4, 10^3, 100 Å, was used with chloroform as an eluent. The sample concentration was 4–5 mg/ml and the flow rate was 0.5 ml/min. The polymer was detected by a Shimadzu (SPD-10A) UV/VIS detector at 254 nm. The calibration was done with polystyrene standards supplied by Polymer Laboratories and Polysciences, Inc. The resulting number-average molecular weight (M_n) is 2.6 kDa and 4.5 kDa for the first block (PBzMA homopolymer) and the final copolymer, respectively. A dispersity, $(D) = M_w/M_n$, where M_w is the weight-average molecular weight, of 1.2 for both, homo and block copolymer was determined by SEC, which agrees with the expected values for this type of polymerisation [26]. The ratio of the two blocks in the copolymer (56:44), was estimated from the integral ratio of the peaks of aromatic and methylene hydrogen at 7.28 and 4.06 ppm, respectively, from the ^{1}H NMR spectrum. The critical micelle concentration (CMC) of poly(benzyl methacrylate)-*block*-poly(2-(dimethylamino)ethyl methacrylate) copolymers as measured by fluorescence spectroscopy and conductivity was found to be in the 10^{-4} to 10^{-3} M range.

2.3. ζ Potential

The ζ-potential of polymersomes and of the SiO_2 particles after each polymersome and PSS assembly were measured with a Malvern NanoSizer (Nano-ZS) (UK). All the ζ-potential measurements