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Layer-by-layer assembly of viral capsid for cell adhesion

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Abstract

Cowpea mosaic virus (CPMV)-based thin films are biologically active for cell culture. Using layer-by-layer assembly of CPMV and poly(diallyldimethylammonium chloride), quantitatively scalable biomolecular surfaces were constructed, which were well characterized using quartz crystal microbalance, UV–vis and atomic force microscopy. The surface coverage of CPMV nanoparticles depended on the adsorption time and pH of the virus solution, with a greater amount of CPMV adsorption occurring near its isoelectric point. It was found that the adhesion and proliferation of NIH-3T3 fibroblasts can be controlled by the coverage of viral particles using this multilayer technique.

Keywords: Cowpea mosaic virus; Layer-by-layer; Self-assembly; Cell adhesion

1. Introduction

Self-assembly of protein cages into higher-order structures offers great opportunities for developing new materials and other applications, including functional membranes, electronics, sensing and energy harvesting [1–9]. Some recent studies have shown that the layer-by-layer (LbL) assembly technique can be readily employed to construct ultrathin films of viruses and other biological particles via electrostatic interactions [9–14]. By controlling the ionic strength and pH of the solution, the LbL assembly technique enables the creation of highly tunable functional surfaces [15,16]. In particular, the LbL assembly process is highly biocompatible and generally does not interfere with the properties and natural architectures of viruses and other biomolecules [9,11,13,14].

Cowpea mosaic virus (CPMV), a plant virus ~30 nm in diameter, has had its physical, biological and genetic properties well characterized over the past few years [17–20]. The virus comprises 60 copies of two protein subunits in an icosahedral symmetry. The particles are remarkably stable, demonstrated by their application in organic reactions and as a model system in bioconjugation chemistry [20,21]. Recent studies have shown the strong interactions between CPMV with some mammalian cells [22,23]. Because the chemical structure of CPMV is well understood and can be readily modified, it is an excellent multivalent platform for tailoring the surface composition and properties to suit particular needs in surface engineering. In particular, it offers an ideal scaffold for investigating how the nanoscale surface morphology and polyvalent ligand display will affect cell response.

Here, the use of CPMV particles as a novel support for cell adhesion and proliferation is explored. By varying the coating density and surface coverage of the viral particles, it is possible to alter cell attachment and outgrowth behavior. In particular, the LbL assembly process used to control the coating density and smoothness of the surface coated with CPMV on quartz probes is thoroughly characterized.

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2. Materials and methods

2.1. Materials

Poly(diallyldimethylammonium chloride) (PDDA) aqueous solution with molecular weight ~100,000–200,000 and poly(styrenesulfonic acid) (PSS) ($M_W = 70{,}000$) were purchased from Sigma–Aldrich Co. All the chemicals were used as received without further purification. Water was purified using a Millipore Milli-Q system (18.2 MΩ).

2.2. Purification of CPMV

CPMV was obtained from cowpea plants. Cowpea plants ~2 weeks old were inoculated with CPMV. The leaves were harvested, and the virus was isolated from the host plant. The leaves were crushed and added to 0.1 M potassium-phosphate buffer at pH 7.8 with 0.2% β-mercaptoethanol. The mixture was centrifuged at 9000 rpm for 15 min before the supernatant was treated with a 1:1 ratio of $CHCl_3$:1-butanol. The aqueous portion was separated, and CPMV was precipitated by the addition of polyethylene glycol 8 K and NaCl. The resultant pellets were re-suspended in 0.1 M potassium-phosphate buffer at pH 7.8. After a final ultracentrifugation at 42,000 rpm for 2.5 h, pure CPMV obtained was re-suspended overnight in 0.1 M potassium-phosphate buffer at pH 7.8.

2.3. Quartz crystal microbalance

AT-cut quartz crystals were manufactured by Beijing Ziweixing Microelectronic Co., Ltd. The frequency was monitored by a Protek Frequency Counter (Model C3100). A crystal 9 mm in diameter was coated on both sides with silver 4.5 mm in diameter, and the resonance frequency was 9 MHz. Before each quartz crystal microbalance (QCM) experiment, the resonator was washed in ethanol solution for 10 min (followed by rinsing with pure water and drying with a stream of N_2). It was then immersed in a PDDA aqueous solution (1 mg ml^{-1}) for 20 min, taken out, washed thoroughly with pure water, and dried with a stream of N_2. The positively charged surface was further immersed in a PSS aqueous solution (2 mg ml^{-1}). This alternate cycle was repeated three times for a precursor film to provide a uniform charge and a smooth surface for subsequent deposition. The electrode with a $(PDDA/PSS)_2$/PDDA precursor film was then alternately immersed in CPMV solution for 20 min and in PDDA solution for 20 min with intermediate water washing and drying. The frequency of QCM was monitored in each adsorption step after drying.

2.4. Other measurements

Tapping-mode atomic force microscopy (AFM) images were obtained in ambient conditions using an SPA300 instrument (Seiko). Si tips with a resonance frequency of ~300 kHz, a spring constant of about 2 N m^{-1} and a scan rate of 0.5 Hz were used. UV–vis spectra of the thin films deposited on quartz slides were collected on a Shimadzu UV-2450 spectrophotometer.

3. Results and discussion

The isoelectric point (p*I*) of CPMV is about 5.5 [24], therefore at neutral pH the viruses can be considered an anionic macromolecule. In the present study, it was found that one interlayer of the cationic polyelectrolyte PDDA was able to induce binding of CPMV to the surface, which is similar to the previous report on the LbL assembly of cowpea chlorotic mottle virus [12]. In a typical experiment, the quartz crystal wafer was cleaned with H_2SO_4:H_2O_2 (7:3) solution, followed by ultrasonication three times in pure water and drying with a stream of N_2. In order to obtain a uniformly charged layer, the quartz wafer was immersed in a PDDA aqueous solution (1.0 mg ml^{-1}) for 20 min, then washed thoroughly with pure water and dried. The positively charged surface was further immersed in a PSS aqueous solution (2.0 mg ml^{-1}). This coating cycle was repeated twice, and a PDDA layer was coated as the outermost surface to denote the precursor film as $(PDDA/PSS)_2$/PDDA, where the numeric value two indicates the number of polymer bilayers. The wafer, as the QCM resonator, was alternatively immersed in CPMV solution (0.1 mg ml^{-1}) for 20 min and PDDA solution (1.0 mg ml^{-1}) for 20 min with steps of washing and drying.

The time-dependent CPMV adsorption on $(PDDA/PSS)_2$/PDDA coated quartz crystal wafer at neutral pH was monitored by QCM. In the QCM experiment, the crystal electrode was coated with Ag on both sides, so the frequency shift came from both sides. As shown in Fig. 1a, the frequency reaches a plateau when the adsorption time is 10 min, which corresponds to 113 ng of CPMV on each side of the QCM electrode calculated by Sauerbrey's equation [25]. The mass increase corresponds to a 76% coverage of the surface of the QCM electrode, assuming monolayer adsorption with spherical packing.

QCM data for CPMV and PDDA depositions are presented in Fig. 1. For each newly adsorbed CPMV layer on the quartz crystal, the average frequency shift is 229 ± 22 Hz, suggestive of a homogeneous CPMV layer. For each adsorbed PDDA layer, the average frequency shift is 35 ± 5 Hz. UV–vis absorbance was also measured to monitor the assembly process. Because PDDA in solution has only slight absorbance in the UV region [26], the UV absorption at a 240–400 nm wavelength of the composite films is primarily attributed to CPMV. Fig. 1c and d show that the absorbance at 260 nm increases linearly with the number of CPMV deposition cycles. Both QCM and UV–vis data indicate the successive deposition of PDDA/CPMV layers.

To document the influence of pH on the LbL process, CPMV solutions at four different pH levels were used to study the pH-dependent deposition behavior. First, the

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