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Supporting Information

for

Virus-like Particles as Virus Substitutes to Design Artificial Virus-Recognition Nanomaterials

Sabine Sykora,[a] Alessandro Cumbo,[b] Gaël Belliot,[c] Pierre Pothier,[c] Philippe F.-X. Corvini[a] and Patrick Shahgaldian[a,*]

[a]School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Muttenz, Switzerland

[b]INOFEA AG, Basel, Switzerland.

[c]Laboratory of Virology, National Reference Center for Enteric Viruses, Public Hospital of Dijon, France.

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Experimental

General

Tetraethyl orthosilicate (TEOS, ≥ 99%), 3-aminopropyl-triethoxysilane (APTES, ≥ 98%), ammonium hydroxide (ACS reagent, 28–30%), ethanol (ACS reagent, anhydrous), glutaraldehyde (Grade I, 25% in water), skim milk and 3,3´,5,5´-Tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich. All chemicals were used without further purification. Norovirus-like particles (NorVLP), made of 180 copies of protein subunits with a mass of 58.96 kDa, were produced and purified as previously described.[1, 2] Monoclonal mouse anti recombinant viral protein 1 (VP1) of Norovirus genogroup II genotype 4 (GII.4) was purchased from Fitzgerald. Monoclonal peroxidase-conjugated horse anti-mouse IgG was purchased from Vector laboratory.

Synthesis of virus-imprinted particles (VIPs)

Silica nanoparticles were synthesized as previously described.[3] Six mL of 3.2 mg mL^{-1} of SNPs were reacted with 4 µL (0.017 mmol) of APTES for 30 min, in glass vials (20 mL) under stirring condition (400 rpm) at 20°C. After two centrifugation-washing cycles with nanopure water, the particles were incubated for 30 min in 6 mL of 1% (v/v) aqueous glutaraldehyde solution. After two centrifugation-washing cycles with water, they were re-suspended in water or in a solution containing the desired concentration of citrate (adjusted at pH 7). The particles were then incubated with the NorVLP (at 0.03 mg mL^{-1}) for 1 h under magnetic stirring. Subsequently, 12 µL (0.054 mmol) of TEOS were added to the reaction mixture and allowed to react for 2 h. The temperature was then lowered to 10°C and 6 µL (0.026 mmol) of APTES were added. Samples were collected at increasing reaction times and washed twice with water. The particles were allowed to stand for 24 h at room temperature for the curing and the stabilization of the organosilica layer. Non-imprinted reference particles were produced in a similar fashion but omitting the VLP addition step. All washing steps were performed by centrifugation at 3,220 g for 5 min and the pellets were re-suspended by ultrasonic treatment for 2 min using an Elmasonic S30H ultrasonic bath.

Template removal

VIPs were suspended in the removal solution (0.1M HCl and 0.01% v/v Triton-X 100) and subjected to an ultrasonic treatment for 10 min at 30°C. Under stirring conditions (600 rpm), the VIPs suspensions were incubated for 30 min at 40°C. Subsequently, the so-treated VIPs were submitted to an additional ultrasonic treatment for 30 min, washed twice water, centrifuged at 3220 g for 5 min and stored at 4°C.

Determination of particle concentration

One mL of particle suspension was freeze-dried and weighted in 1.5 mL tube. The dry mass was determined through subtraction of the mass of the empty tube from the mass of the tube containing the dried particles.

Scanning electron microscopy and particle size measurement

Particles were imaged using a Zeiss SUPRA 40VP scanning electron microscope. Two µL of sample were spread on freshly cleaved mica sheets, dried at ambient conditions and sputter-coated with a gold–platinum alloy for 15 s at 10 mA (SC7620 Sputter coater). Micrographs were acquired using the InLens mode with an accelerating voltage of 20 kV. Particle sizes were measured on micrographs acquired at a magnification of 150,000 X using the Olympus Analysis software package. More than 100 measurements were made per type of particles.

Virus-binding assay

A solution containing 65 pM NorVLP, 10 mM sodium phosphate, 10 mM citrate, 50 mM NaCl and 75 mg mL^{-1} of skim milk (final pH 6) was mixed with the particles (either VIPs or NIPs) at a concentration of 834 mg mL^{-1} in a final volume of 120 µL. Reference solutions containing the same amount of NorVLP were prepared by omitting the particle addition step. The interaction assays and reference solutions were incubated at 25°C under shaking at 650 rpm. After the desired contact time, the suspension was centrifuged at 16100 g for 1 min and 100 µL of the supernatant was withdrawn for virus quantification by an indirect ELISA assay (see next section). The measured values from the interaction assays were compared with the values obtained from reference solutions, which were prepared and treated in the same fashion than the interaction assays.

Detection of NorVLP

The detection of NorVLP was carried out with an indirect ELISA assay. The supernatant of the interaction assay containing unbound NorVLP were diluted (1:8) with carbonate-bicarbonate buffer (0.2 M, pH 9.6) and then coated on a 96-well microplate (Nunc Maxisorp, ThermoScientific) at 4°C overnight, in duplicate. The microplate was washed three times with phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween-20 using a microplate washer ELx50 (BioTek). After adding 200 µL of skim milk (4% in PBS), the plates were incubated for 2 h at 37°C and then washed as described before. Norovirus VP1 antibody was diluted 1:10,000 in PBS and added to the wells (100 µL per well). After incubation at 37°C for 1 h, the wells were washed as described above. The secondary peroxidase-conjugated antibody was diluted 1:10,000 with skim milk (4% in PBS) and added to the wells (100 µL per well). After incubation at 37°C for 30 min, the wells were washed as described above. After adding 100 µL TMB substrate into each well, the plate was incubated at room temperature in the dark for 15 min. The reaction was stopped through the addition of 50 µL per well of stop solution (1M HCl). The optical density was measured at 450 nm using a Synergy H1 Hybrid Reader (BioTek). Negative controls were skim-milk-blocked wells without NorVLP and NorVLP-coated wells without the addition of Norovirus VP1 antibody. The average optical density from all negative controls was subtracted from the optical density from experimental wells.

Of note, PBS contained 0.2 g L^{-1} of potassium chloride, 0.2 g L^{-1} of potassium phosphate monobasic anhydrous, 8 g L^{-1} of sodium chloride and 1.15 g l^{-1} of sodium phosphate dibasic in water (final pH 7.5).

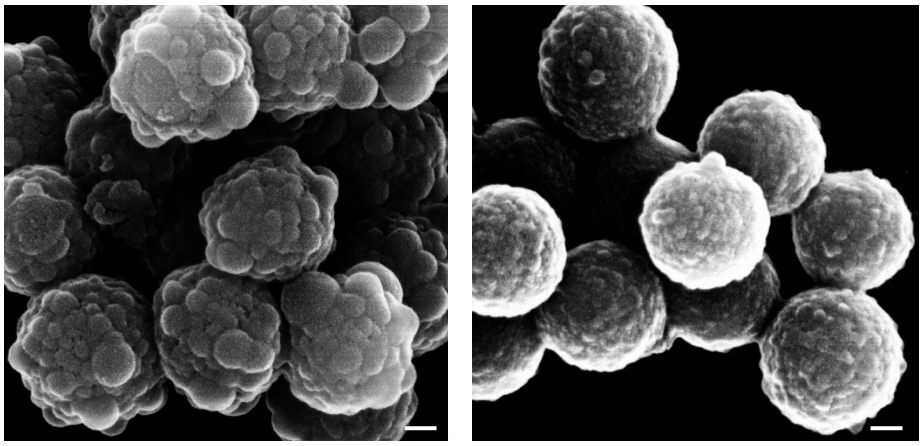

Fig. S1 Effect of sonication on VIP surface; FESEM micrographs of VIPs (after 6 h silane polycondensation in nanopure water) before (left) and after (right) sonication for 20 min; scale bar: 100 nm.

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