Supporting Information

Digital Light Processing (DLP) 3D Printing of Polymer Networks Comprising Virus-like Particles†

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Experimental section

Expression and preparation of PhMV VLPs

The 564-bp PhMV coat protein gene (NCBI Gene ID 940246) was inserted into pET and pRSETa bacterial expression vectors using established methods (Gibson DG et al., Nature Methods, 2009). The resulting pET-PhMV CP and pRSETa-PhMV CP vectors were used to double transform BL21(DE3) cells (NEB). A single colony was isolated and used to inoculate Lysogeny broth supplemented with 50 μg/mL carbenicillin and 50 μg/mL kanamycin, which was incubated for 18 hours at 37ºC with shaking (225 rpm). Glycerol (Sigma-Aldrich) was added to a final concentration of approximately 25 % (v/v) and the resulting solution was aliquoted and stored at -80ºC. The expression and purification of PhMV VLPs was then carried out as previously reported (Sastri M et al., J Mol Biol, 1997). A small amount of BL21 cells in glycerol stock containing pRSETa-PhMV CP and pET-PhMV CP were inoculated into 50 mL of lysogeny broth (LB) containing 50 μg/mL of carbenicillin and 50 μg/mL kanamycin and the medium was incubated for 18 h (37 °C, 225 rpm). 10 mL of the preculture were then inoculated into 500 mL of Terrific Broth (TB) also containing 50 μg/ml each carbenicillin and kanamycin and left to incubate (37ºC, 250 rpm). When culture density reached OD_{600}~1 (approximately 8 hours), protein expression was induced with 0.5 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG; Gold Biotechnology), and the cells were incubated at 30°C overnight. The culture was then centrifuged (7500 x g, 10 min, 4°C), the pellet was resuspended in 50 mM sodium citrate buffer (SCB) and the resulting suspension was sonicated (10 min, 30% amplitude, 5 sec ON, 2 sec OFF) and centrifuged (10000 x g, 10 min, 4°C). The lysates were centrifuged (27000 x g, 30 min, 4°C), and the supernatant was subjected to ultracentrifugation (35000 rpm, 50.2 Ti rotor, Beckman Coulter Inc., 3h, 4°C). The resulting pellets were resuspended in SCB overnight. Insoluble material was removed by centrifugation (15000 x g, 10 min, 4°C). Supernatant was extracted with 1:1 $CHCl_3$/ButOH and the aqueous layer was isolated by centrifugation (5000 x g, 10 min, 4°C). The resulting solution was centrifuged (15000 x g, 10 min, 4°C) to remove insoluble material. The suspension was then layered carefully onto a 10−40 % (w/v) linear sucrose gradient and ultracentrifuged (28000 rpm, SW32 rotor, Beckman Coulter Inc., 3h, 4°C). The light scattering fractions were collected and pooled with syringe metal needle, diluted in SCB 1:1, and concentrated in ultra-centrifuge (42000 rpm, 50.2 Ti rotor, Beckman Coulter Inc., 3h, 4°C). The clear colorless pellet was finally resuspended in PBS and stored at 4°C. The VLP concentration was measured using the bicinchoninic acid (BCA) assay using bovine serum albumin (BSA) as a standard.

VLP resin (VLP-R) synthesis and characterization

In this work 3 resins were analyzed, all of them with 20 wt% solids, including PhMV and polyethylene glycol diacrylate (PEGDA) and 80 wt% liquid including PBS, DCM, and LAP solution. A PhMV solution in PBS (0.1, 0.5 and 1 wt%) was mixed with PEGDA Mn = 575 (19.9, 19.5, and 19.0 wt% respectively) in a solution of 12 %wt DMSO and 67 wt% PBS and the reaction was left for a 24h period at room temperature with stirring. To characterize the VLP resins, the excess PEGDA was removed by ultracentrifugation (121, 139g, 70 min, 4°C, over a 20% sucrose cushion in PBS). The resulting pellet was resuspended in PBS and characterized. For 3D-printing, purification via ultracentrifugation was not carried out, instead 1 wt% LAP was added and mixed into the slurry resin mixture before transferring them to the DLP printer.

Native and Denaturing Gel Electrophoresis

Denatured CP (5 μg per lane) were analyzed by polyacrylamide gels electrophoresis using 12% NuPAGE gels and 1× MOPS buffer (Invitrogen). Samples were denatured by boiling in 1:10 reducing agent and 1:4 SDS blue loading dye for 5 min. VLPs (5 μg per lane) were analyzed on 0.8% (w/v) agarose native gel electrophoresis in 0.1 M Tris-borate-EDTA (TBE) running buffer (pH 8.3). All gels were stained in Coomasie blue staining documented by photography under UV light in a FluorChem R system (Bio-techne).

Size Exclusion Chromatography (SEC)

VLPs were analyzed by SEC using a Superose-6 increase 10/300 GL column (P_i = 5 MPa, ΔP = 5 MPa) on the Äkta Pure system (GE Healthcare). The column was loaded with 250 μL sample (1.4 mg/mL for VLP, VLP-R-1 and VLP-R-0.5 and 0.4 mg/ml for VLP-R-0.1) and the sample was run for 50 min at a flow rate of 0.5 mL/min in PBS buffer.

Dynamic Light Scattering

DLS analysis was performed in a Zetasizer Nano ZSP/Zen5600 instrument (Malvern Panalytical, Malvern, UK). All VLP samples were measured at a concentration of 0.4 mg/ml in PBS.

Rheology

Rheological measurements were performed on a TA Instruments Discovery Hybrid Rheometer-2. Both viscosity and potting tests were performed on the resins to determine their suitability for vat 3D printing. Viscosity versus shear rate tests were carried out in a shear rate range from 1 to 100 S^{-1}. Photorheology tests to determine the cure rate were carried out at a Strain of 1%, a frequency of 1 Hz, and a light intensity of 20 W/s. A 30 s dwell time elapsed before the UV lamp was turned on for 1 min. Both tests were carried out with 20 mm parallel plates with a separation height of 1000 μm.

3D printing of VLP bioplastics (VLP-BP)

An Asiga Max Mini printer with a LED source centered at 405 nm was used to fabricate the 3D-printed constructs, while the build plate and resin tray were modified to reduce the total volume of resin required down to 25 X 25 mm. The 3D objects designed by 3 Autodesk Fusion 360 or downloaded from Thingiverse were prepared for printing in the Asiga Composer software. The optimized printing parameters were a burn-in layer of 0.5 s, an exposure time of 0.3 s, and a layer thickness of 50 μm at an intensity of 20 mW/cm^2. To determine these parameters, two types of structure were used, one consisting of lines of different thicknesses and another structure consisting of gaps and heights. The printed objects were washed with DI water and dried with air to clean the uncured resin from the surface.

Table S1. Hydrodynamic radii (R_H) and Polydispersity index (PDI)

Entry	R_H (nm)	PDI
VLP	32.7	0.232
VLP-R-0.1	37.8	0.123
VLP-R-0.5	43.8	0.141
VLP-R-1	32.7	0.246

Bioplastic Formulation

The 3D printed objects were air-dried for 48 h. Then, the bioplastics were subjected to isothermal heating at 100°C for 30 min to determine the degree of hydration. When the water content was found to be 1% or less (see Figure S1), it was assumed that the bioplastics were effectively dry. Consequently, the percentages of PEGDA and VLP in the bioplastic were calculated.

For example, if we consider a formulation in which the total bioplastic content is 20% (with 80% water content) and if we consider a VPL-R-1 resin as an example, the bioplastic component thus is composed of 5 wt% VLPs and 95 wt% PEGDA.

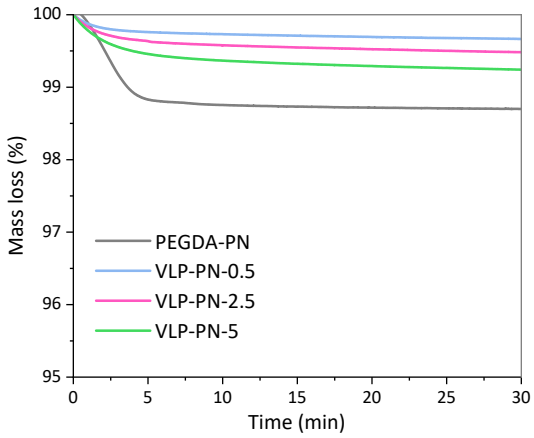

Figure S1. Mass loss rates vs time for VLP-PN samples (0.5-5%) and the native PEGDA network under the isothermal heating condition at 100 °C.

Thermogravimetric Analyzer (TGA)/ Differential Scanning Calorimeter

Thermogravimetric analysis (TGA) was performed by analyzing bioplastics samples (15-30 mg) or lyophilized VLPs (2.3-3.6 mg) on a Discovery SDT 650™ (TA Instruments™) simultaneous DSC/TGA. Samples were equilibrated at 50°C, then ramped up at 10°C/min until 1200°C under N_2 atmosphere. All samples were carried out in duplicate.

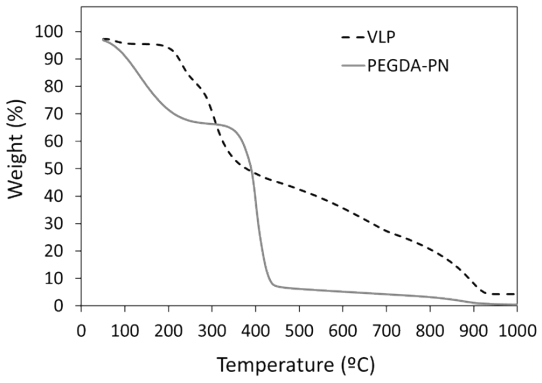

Figure S2. Thermograms of VLPs and PEGDA-PN.

Mechanical Properties

TestResources Universal Test System load frame was used to analyze the mechanical properties at a constant rate of 5 mm/min until failure with a 1KN Load Cell. Dogbones followed the ISO 527-2 specimen specifications. The Dogbones were prepared by adding the resin into a Teflon mold, with a glass slide placed on top to have perfectly flat dogbones and curing into a UV box for 2 mins. Then the samples were removed from the mold and dried for 48 h before tested.

Table S2. Resin Formulation

Name	VLP (%)	PEGDA (%)	LAP (%)	PBS	DMSO
PEGDA	0	20	1	50.15	8.85
VLP-R-0.1	0.1	19.9	1	50.15	8.85
VLP-R-0.5	0.5	19.5	1	50.15	8.85
VLP-R-1	1	19	1	50.15	8.85

Table S3.Bioplastic Formulation

Name	VLP (%)	PEGDA (%)
PEGDA-BP	0	100
VLP-R-0.1	0.5	99.5
VLP-R-0.5	2.5	97.5
VLP-R-1	5	95

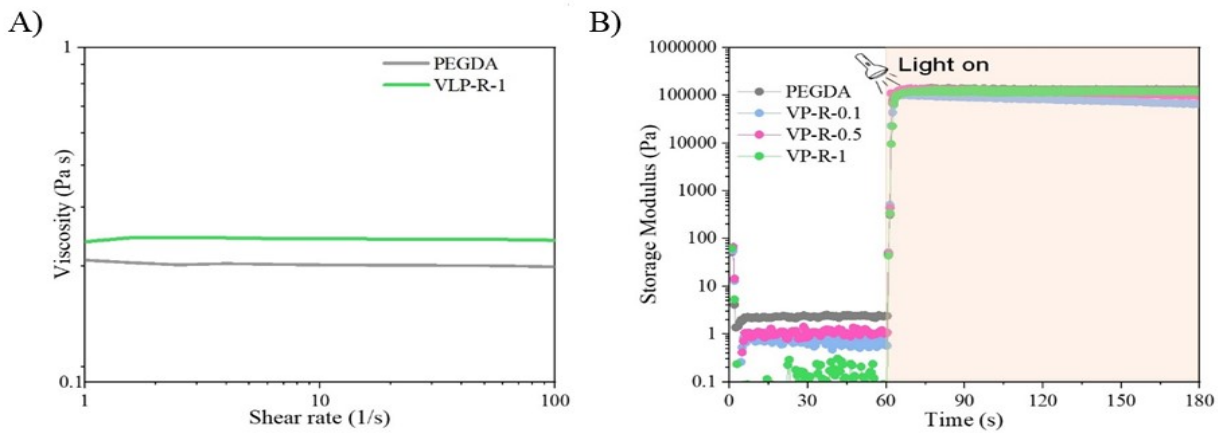

Figure S3. Rheometry of VLP resin formulations to determine viscosity and rate of photocuring. (a) Viscosity versus shear rate data for neat PEGDA resin (0 wt% VLP) and VLP-R-1 resin (1 wt% VLP). (b) Photorheometry, for VLP-R samples (VLP-R-0.1, VLP-R-0.5 and VLP-R-0.1 with 0.1, 0.5 and 1 wt% VLP weight contents, respectively) and neat PEGDA resin (0 wt% VLP) to analyze the rate of photocuring for all resins. The light source (400 nm I=20mW cm^{-2}) was turned on after 60 s.