Electronic Supplementary Information (ESI)

Preparation of hyaluronic acid-coated polymeric micelles for nasal vaccine delivery

Kengo Suzuki[1], Yuta Yoshizaki[2†], Kenta Horii[1], Nobuo Murase[2], Akinori Kuzuya[1,3], Yuichi Ohya[1,3]*

[1] Faculty of Chemistry, Materials, and Bioengineering, Kansai University, 3–3–35 Yamate, Suita, Osaka 564–8680, Japan

[2] Organization for Research and Development of Innovative Science and Technology (ORDIST), Kansai University, 3–3–35 Yamate, Suita, Osaka 564–8680, Japan

[3]Kansai University Medical Polymer Research Center (KUMP-RC), Kansai University, 3–3–35 Yamate, Suita, Osaka 564–8680, Japan

†Present address: Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8578, Japan

***Corresponding author: Yuichi Ohya**

e-mail: yohya@kansai-u.ac.jp, TEL +81-6-6368-0818, FAX: +81-6-6339-4026

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Preparation of PLys$^+$-*b*-PLLA

Materials

L-Lactide, 1-dodecanol, $Sn(Oct)_2$, *N*-(*tert*-butocycarbonyl)glycine (Boc-Gly-OH), *N,N'*-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), trifluoroacetic acid (TFA), triethylamine (TEA), 25% HBr/AcOH, diethyl ether, chloroform, methanol, *N,N*-dimethylformamide (DMF), chloroform-*d* ($CDCl_3$), dimethyl sulfoxide (DMSO)-*d*6 and other reagents were purchased from Fujifilm Wako Pure Chemical Corporation (Japan). *N*-Benzyloxycarbonyl-L-lysine N-carboxy anhydride (Lys(Z)-NCA) was purchased from Hodogaya Contract Laboratory Co. Ltd. (Japan). Hyaluronic acid sodium salt (Mw = ~ 90,000 Da) was obtained from Kibun Food Chemifa Co., Ltd. (Japan). Water was purified using Millipore Elix UV3 direct-Q UV (Merck, Darmstadt, Germany).

Measurements

^{1}H-Nucleic magnetic resonance (NMR) spectra were obtained on a JNM-ECS400 or GSX-400 spectrometer (JEOL, Tokyo, Japan). The molecular weight was measured by size-exclusion chromatography (SEC) (Tosoh GPC-8020 series system; Tosoh TSKgel α-5000 or Tosoh GMH_{HR}-H(S), Tosoh Corporation, Tokyo, Japan).

Synthesis of polylactide with amino-terminal (H_2N-PLLA)

We synthesised polylactide with an amino-terminal (H_2N-PLLA) by bulk ring-opening polymerisation of L-lactide, condensation with Boc-Gly-OH, and subsequent deprotection according to previous literature[36,37].

First, L-lactide (69.4 mmol, 10.0 g), 1-dodecanol (2.31 mmol, 430 mg), and $Sn(Oct)_2$ (69.4 μmol, 28.1 mg) were placed in a flask with stopcock and dried under reduced pressure for 6 hours. After melting in an oil bath at 150°C, bulk ring-opening polymerisation was carried out at 120°C for 9 h. The reaction mixture was dissolved in a small amount of chloroform and re-precipitated using a mixture of chloroform and diethyl ether (1:20). The precipitate was collected via suction filtration and dried under reduced pressure. Polylactide with free OH terminal (and dodecyl ester terminal) (HO-PLLA) was obtained as a white solid (yield: 88%). ^{1}H-NMR spectrum is shown in **Figure S1**. M_w = 7,100, M_n = 5,500, M_w/M_n: 1.30 estimated by SEC.

Boc-Gly-OH (3.0 mmol, 0.53 g) and DCC (3.7 mmol, 0.76 g) were separately dissolved in dry methylene chloride (2.17 mL for Boc-Gly-OH and 3.05 mL for DCC). The DCC solution was added dropwise to the Boc-Gly OH solution, and the mixture was stirred for 1 h in an ice bath. In another flask, HO-PLLA (0.75 mmol, 3.00 g) and DMAP (0.41 mmol, 50.0 mg) were dissolved in dry methylene chloride (20.5 mL). The solution of HO-PLLA and DMAP was added to the Boc-Gly-OH and DCC solution at room temperature. The mixture was stirred for 24 h, and the dicyclohexylurea residue was removed by suction filtration. The product (Boc-NH-PLLA) was dissolved in a small amount of chloroform and re-precipitated using chloroform and diethyl ether (1:20). The precipitate was collected via suction filtration and dried under reduced pressure (Yield: 81%). ^{1}H-NMR spectrum is shown in **Figure S2**. M_w = 6,500, M_n = 5,700, M_w/M_n = 1.14 estimated by SEC.

Boc-NH-PLLA (542 μmol, 2.21 g) in a flask was dried for 4 h under reduced pressure and dissolved in dry methylene chloride (15 mL) under a nitrogen atmosphere. TFA (81 mmol, 6.2 mL) was then added to the flask and stirred for 1 h at 0 C in an ice bath. After the reaction, reprecipitation was performed three times with chloroform and hexane (1:20). A white solid was obtained after drying under reduced pressure. The product (H_2N-PLLA TFA salt) was dissolved in dry methylene chloride (12 mL), and TEA was added to the solution in an ice bath for desalting. The reaction mixture was stirred for 1 min. Reprecipitation was performed thrice with chloroform and hexane (1:20).

After drying under reduced pressure, H_2N-PLLA was obtained as a white solid (yield: 79%). The ^1H NMR spectrum is shown in **Figure S2**. M_w = 5,700, M_n = 5,000, M_w/M_n = 1.31 estimated by SEC.

Synthesis of PLys$^+$-b-PLLA

The synthesis of the diblock copolymer of poly(L-lysine) and polylactide, PLys$^+$-*b*-PLLA, was carried out by ring-opening polymerisation of Lys(Z)-NCA using the obtained H_2N-PLLA as a macro-initiator and subsequent deprotection according to a method reported previously[36,37].

H_2N-PLLA (386 μmol, 1.35 g) and Lys(Z)-NCA (11.6 mmol, 3.54 g) were dried at reduced pressure for 7 h. H_2N-PLLA was dissolved in dry chloroform (20.2 mL) in two-necked flasks and stirred in an oil bath at 40°C. Then, a Lys(Z)-NCA solution in chloroform (3.4 mL) was added to initiate the polymerisation. The reaction was carried out at 40°C for 24 h under a nitrogen atmosphere. After the reaction, the products were reprecipitated three times using a mixture of diethyl ether and methanol (10:1), owing to the poor solubility of the product in this mixture. The precipitate was collected via centrifugation and dried under reduced pressure to obtain a white solid of PLys(Z)-*b*-PLLA (yield: 70%). ^1H-NMR spectrum is shown in **Figure. S3**. M_w = 13,000, M_n = 10,000, M_w/M_n = 1.3 estimated by SEC.

The benzyloxycarbonyl(Z) group of PLys(Z)-*b*-PLLA was removed as follows: PLys(Z)-*b*-PLLA (56.7 μmol, 681 mg) was dried under vacuum overnight and then dissolved in TFA (6.1 mL) in a flask equipped with a calcium chloride tube. HBr/AcOH (25%, 18.7 mmol, 4.4 mL (10 eq of Z group)) was added to this solution. The ice-cooled reaction mixture was stirred at 0°C for 30 min. After the reaction, the products were precipitated with diethyl ether. The precipitate was collected *via* centrifugation and dried under reduced pressure to obtain a white solid (yield: 80%). The degree of deprotection of the Z group was estimated to be 96% using ^1H-NMR spectroscopy. ^1H-NMR spectrum is shown in **Figure. S3**. M_w = 9,000, M_n = 7,500, M_w/M_n = 1.20 estimated by SEC.

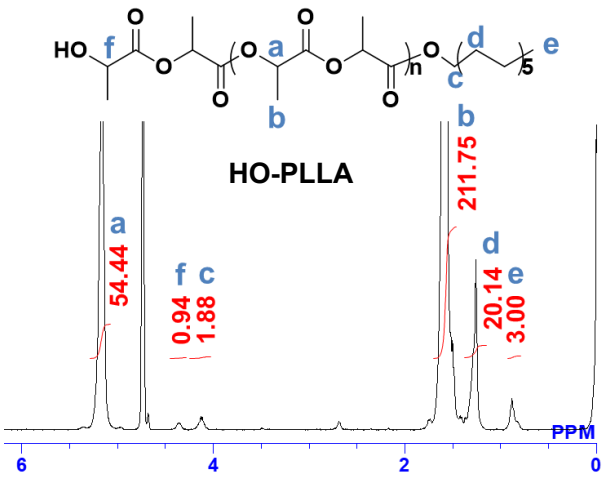

Figure S1. ^{1}H NMR spectrum of HO-PLLA. (Solvent: $CDCl_3$)

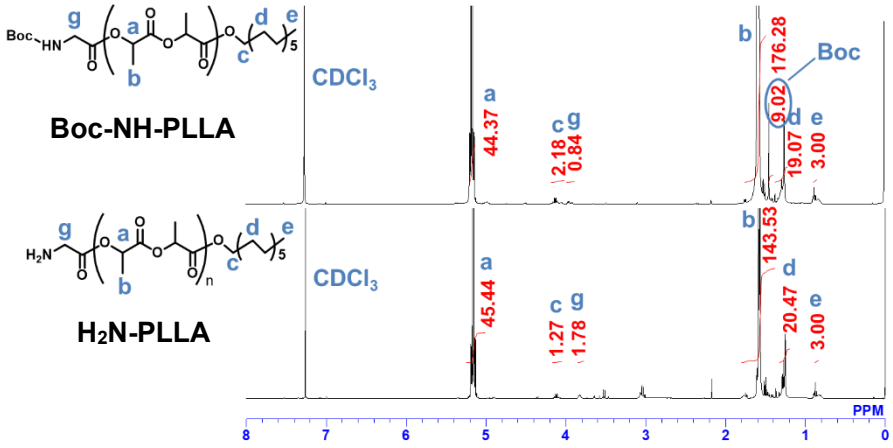

Figure S2. ^{1}H NMR spectra of Boc-NH-PLLA and H_2N-PLLA. (Solvent: $CDCl_3$)

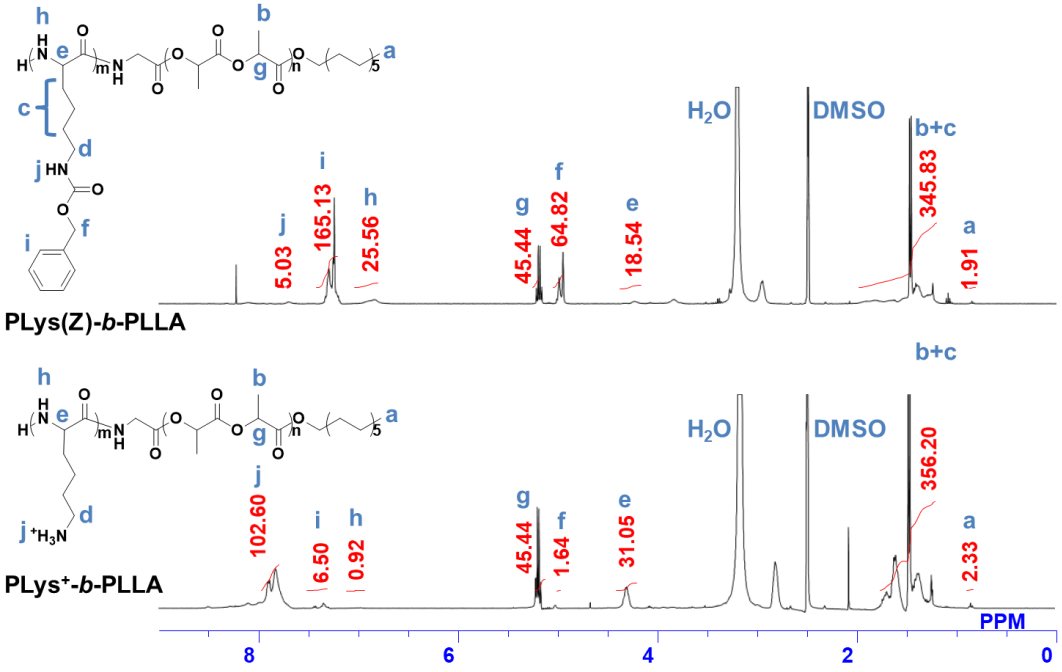

Figure S3. ^{1}H NMR spectra of PLys(Z)-*b*-PLLA and PLys^{+}-*b*-PLLA. (Solvent: $CDCl_3$)

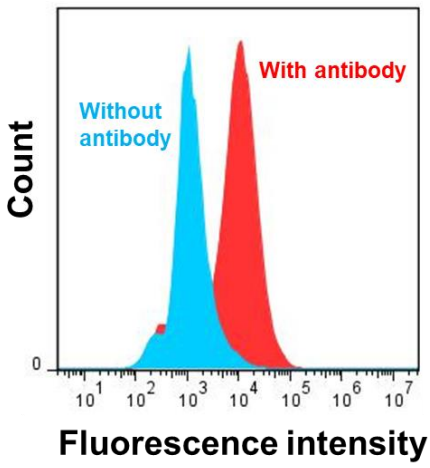

Figure S4. Quantification of dendritic cell markers (CD11c^{+}) of the collected bone marrow cells using a flow cytometer after treatment with R10 medium containing 10 ng/mL GM-CSF.

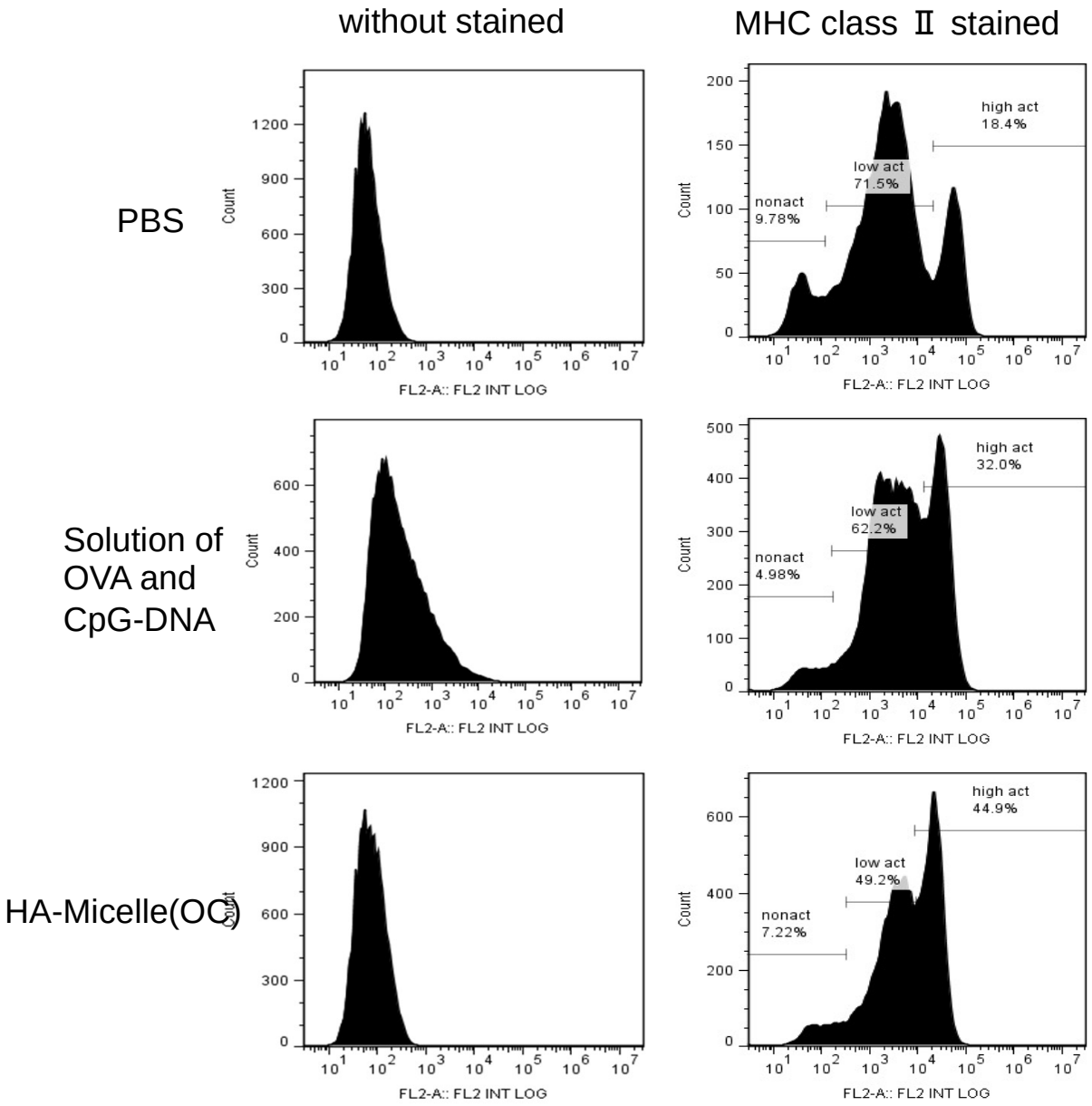

Figure S5. FCA of BMDCs with or without PE-labelled anti-MHC class II antibody staining. Cells were incubated with HA micelles (OC), an aqueous solution of OVA and CpG-DNA or PBS in RPMI-1640 containing 5% FBS for 24 h at 37°C.

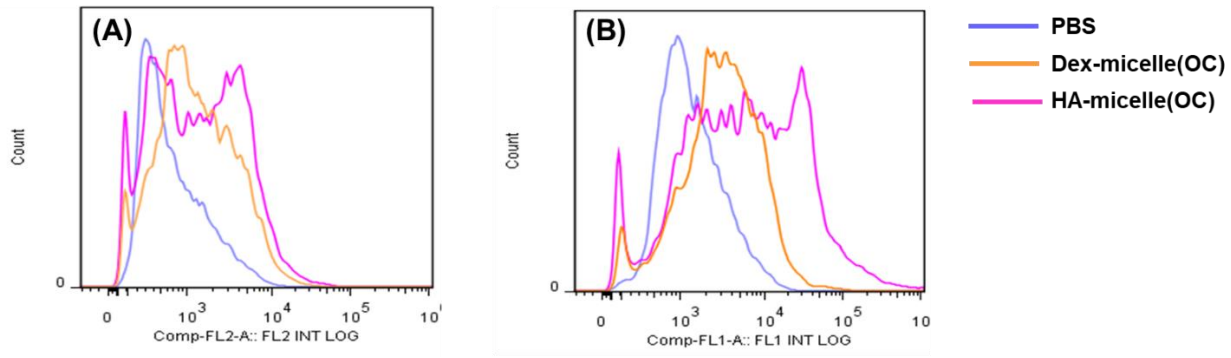

Figure S6. Flow cytometric analysis (FCA) for BMDCs incubated with HA-micelle(OC) or Dex-micelle(OC) containing Rhodamine-labelled OVA and FAM-labelled CpG-DNA at 37°C for 4 h. (A) Rhodamine B detection (OVA), (B) FAM detection (CpG-DNA).

Table S1. Sequences of qPCR primers

Primers		Sequences	References
IL-4	forward	5'-ATGGGTCTCAACCCCCAGCTAGT-3'	[1]
	reverse	5'-GCTCTTTAGGCTTTCCAGGAAGTC-3'	
IFN-γ	forward	5'-TGAACGCTACACACTGCATCTTGG-3'	[2]
	reverse	5'-CGACTCCTTTTCCGCTTCCTGAG-3'	
β-Actin	forward	5'-TGGAATCCTGTGGCATCCATGAAA-3'	[2]
	reverse	5'-TGAACGCTACACACTGCATCTTGG-3'	

[1] M. Galdiero, A. Marcatili, G. Cipollaro del'ero, I. Nuzzo, C. Bentivoglio, M. Galdiero, C. R. Carratelli, Effect of transforming growth factor β on experimental *Salmonella typhimurium* infection in mice, *Infect. Immun*., **1999**, *67*, 1432-1438.

[2] L. Huang, F. Chen, Y. Chen, Y. Lin, J. T. Kung, Potent induction of long-term $CD8^+$ T cell memory by short-term IL-4 exposure during T cell receptor stimulation, *Proc. Natl. Acad. Sci. USA*., **2000**, *97*, 3406-3411.