One-Pot Synthetic Route to Polymer-Silica Assembled Capsule Encased with Nonionic Drug Molecule

You-Hwan Son, Man park, Young Bin Choy, Hye Ryung Choi, Dong Seok Kim, Kyoung Chan Park, and Jin-Ho Choy*

Experimental

Material: All materials were of analytical grade and used without any further purification. TEOS, Crystal violet, synthetic melanin, and α-MSH were obtained from Sigma-Aldrich Co. Pluronic F127 was purchased from BASF. C2-ceramide was obtained from Avanti polar-lipids.

Sample preparation: Pluronic F127 and C2-ceramide were dissolved in a 10% ethanol solution with vigorous stirring at 60 °C. The solution pH was adjusted to 2 by adding 0.1M hydrochloric acid. While vigorously stirred, TEOS was added dropwise, and then the resulting gel was aged at 60 °C for 24 h. Finally, the precipitate was filtered, washed, and dried at 60°C in vacuum. The starting molar composition was x F127: y C2-ceramide: 1.0 TEOS: 4.0HCl: 15 EtOH: 117 H2O. Thus obtained samples were denoted as DPS-AS (x = 0.0035, y = 0), and DPS-C2 ( x = 0.0020, y = 0.0015 ), respectively. In order to know loading capacity of C2-ceramide, the encased C2-ceramide was separated by solvent extraction method.1 The DPS-C2 (0.01g) was suspended in MeOH (50 ml) solution and stirred at 60 °C for 4 h, and filtered to remove mesoporous silicas. Finally, the filtrate was dissolved in chloroform (50 ml) and then the MeOH was removed using a separating funnel for HPLC measurement.

Cell cultures: B16 murine melanoma cells were obtained from the Korean Cell Line Bank
(Seoul, Korea). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA) and 1% penicillin-streptomycin (10,000 U/ml and 10,000g/ml, respectively) in 5% CO₂ at 37°C.

**Cell viability assay:** Cell viability was examined by the crystal violet assay.² The cells were incubated in the media with various concentrations of DPS-C2 (1, 10, 20, 30, 40 μg/mL) for 24 hrs. Afterwards, the medium was removed and stained with 0.1% crystal violet in 10% ethanol for 5 min at room temperature and rinsed four times with distilled water. Crystal violet retained by the adherent cells was extracted with 95% ethanol, whose absorbance was determined at 590 nm using an ELISA reader (TECAN, Salzburg, Austria).

**Measurement of melanin contents:** Extracellular melanin was measured as described previously³ but with a slight modification. Briefly, the B16 cells were incubated with a density of 1 × 10⁵ cells in six-well plates overnight. In the presence of α-MSH (1 uM), the cells were treated with the known concentrations of DPS-AS (silica, 38 μg/mL), DPS-C2 (38 μg/mL), and C₂-ceramide (10 uM) as a positive control in phenol red free DMEM for 3 days at 37 °C, respectively. Optical densities (OD) were measured at 400 nm using an ELISA reader. A standard curve for synthetic melanin (0 ~ 300 μg/ml) was prepared in triplicate for each set of the experiments. The cells were then counted with a haemocytometer.
Characterization: SAXS patterns were obtained at 200 mA, 50kV with Rigaku DMAX 2500. Field emission-scanning electron microscopic (FE-SEM) was monitored with a JEOL JSM-6700. TEM images were obtained with Philips CM200 operating at an acceleration voltage of 200 kV. Infrared spectra were measured with Jassco 660 FT–IR spectrometer by the standard KBr disk method. C\textsubscript{2}-ceramide content was quantitatively measured by HPLC–ELSD system with a PU 980 pump and an evaporative light scattering detection system (alltech 2000) under the following condition: evaporative temperature, 80 °C; gain, 8; gas, 1.9 bar nitrogen.

Reference

**Figure S1:** FT-IR Spectra of (a) DSP-C2, (b) C\textsubscript{2}-Ceramide and (c) F127.
For DPS-C2, those characteristic bands corresponding to amide modes were shifted slightly and appeared at 1642 cm$^{-1}$ (amide I) and 1550 cm$^{-1}$ (amide II), which may explain a weak intermolecular interaction among C$_2$-ceramide molecules. These band shifts indicated that C$_2$-ceramide and were homogenously incorporated with each other.

**Figure S2:** TG & DTA of (a) DSP-AS and (b) DSP-C2.
Three-step thermolysis was seen from Thermogravimetric (TG) analysis with a total weight loss of 52.6 wt %. Differential thermal analysis (DTA) exhibited one endothermic and two exothermic peaks. The endothermic loss around at 80°C (2.5 wt % loss) was assigned to the dehydration (a), but two exothermic weight losses at 180 °C and 310 °C were due to the desorption and decomposition of a templating surfactant.\textsuperscript{[18]} In case of DPS-C2, however, a total weight loss was determined to be 41.4 wt% with one endothermic and three exothermic peaks. The second exothermic peak at 220°C could be assigned to the decomposition of C2-ceramide, which was different from that of DPS-AS (b).

\textbf{Figure S3:} Nitrogen adsorption isotherms for (a) DSP-AS and (b) DSP-C2

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Figure S4: The percentile release of $C_2$-ceramide versus time for the DPS-C2 (●) and the sample (○) where the $C_2$-ceramide molecules were inserted into empty pores of
The release profile was obtained as described by M. Vallet-Regí et al.* The release from DPS-C2 was fast during the first 8 h and became slow afterwards. The maximum amount of release, 91%, was obtained at 36 h. For the sample with conventional drug loading, however, the release rate was much slower than that of DPS-C2. The maximum amount of release was only 61%. Although both samples exhibited sustained release property, the release from DPS-C2 appeared to be more efficient due to the enhanced water dispersibility by Pluronic F127.


Figure S4: Effects of DPS-C2 on B16 cell viability. Cells were serum-starved for 24 hrs
and DPS-C2 was treated in serum-free media at 1-40 g/mL for 24 hrs.

**Figure S5**: Effects of DPS-C2 on melanogenesis in B16 cells. In the presence of α-MSH (1
μM), the cells were cultured with 10-30 μg/mL of DPS-C2 for 3 days.