Supplementary Material (ESI) for Chemical Communications

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

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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. C_2-ceramide was obtained from Avanti polar-lipids.

Sample preparation: Pluronic F127 and C_2-ceramide were dissolved in a 10% ethanol solution with vigorous stirring at 60 ^oC. 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 ^oC for 24 h. Finally, the precipitate was filtered, washed, and dried at 60^oC in vacuum. The starting molar composition was x F127: y C_2-ceramide: 1.0 TEOS: 4.0HCl: 15 EtOH: 117 H_2O. 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 C_2-ceramide, the encased C_2-ceramide was separated by solvent extraction method.[1] The DPS-C2 (0.01g) was suspended in MeOH (50 ml) solution and stirred at 60 ^oC 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% CO_2 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).

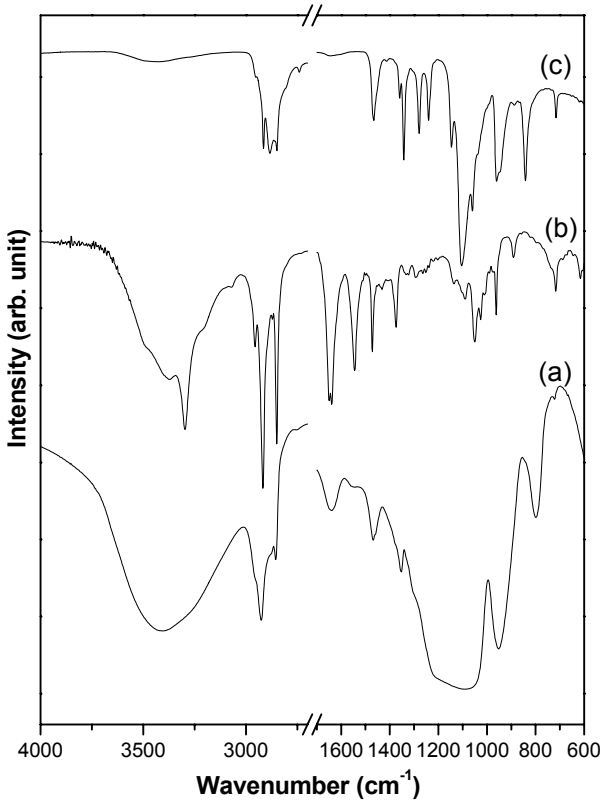
Measurement of melanin contents: Extracellular melanin was measured as described previously[3] but with a slight modification. Briefly, the B16 cells were incubated with a density of 1×10^5 cells in six-well plates overnight. In the presence of α-MSH (1 uM), the cells were treated with the known concentrations of DPS-AS (silica, 38 μg/mL), DPS-C2 (38 μg/mL), and C_2-ceramide (10 uM) as a positive control in phenol red free DMEM for 3 days at 37 ^oC, 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_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 ^oC; gain, 8; gas, 1.9 bar nitrogen.

Reference

1 M. Kruk, M. Jaroniec, C. H. Ko, R. Ryoo, *Chem.Mater.* 2000, **12**, 1961.
2 T. P. Dooley, R. C.Gadwood, K. Kilgore, L. M. Thomasco, *Skin Pharmacol.* 1994, **7,** 188.
3 K. Smalley, T. Eisen, *FEBS Lett,* 2000, **476**, 198.

Figure S1: FT-IR Spectra of (a) DSP-C2, (b) C_2-Ceramide and (c) F127.



: ν_{max}/cm^{-1} (a) 3450 (OH), 2917 and 2850 (CH), 1642 (amide I), 1550 (amide II); (b) 3450 (OH), 3297 (NH), 2956, 2917 and 2850 (CH), 1649 and 1641 (amide I), 1541 (amide II), 1470 (CH, scissoring); (c) 2956 and 2917 (CH) 1465 (CH, scissoring).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 an weak intermolecular interaction among C_2-ceramide molecules. These band shifts indicated that C_2-ceramide and were homogenously incorporated with each other.

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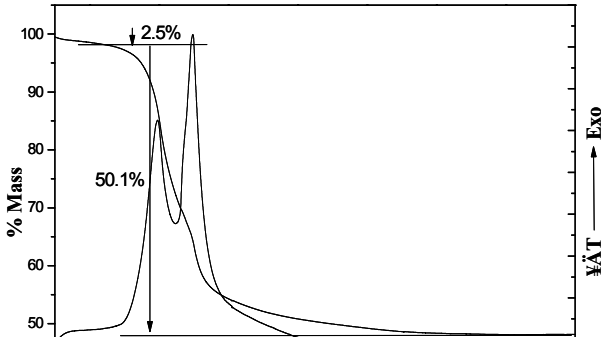
Figure S2: TG & DTA of (a) DSP-AS and (b) DSP-C2.



(a)

(b)

: 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^{o}C (2.5 wt % loss) was assigned to the dehydration (a), but two exothermic weight losses at 180 ^{o}C and 310 ^{o}C were due to the desorption and decomposition of a templating surfactant.[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^{o}C could be assigned to the decomposition of C_2-ceramide, which was different from that of DPS-AS (b).

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

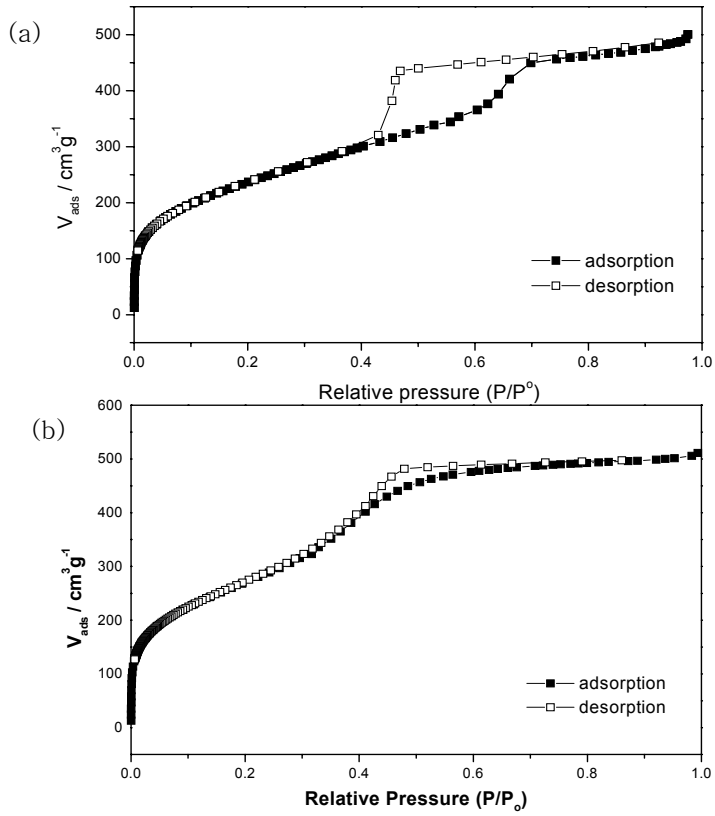

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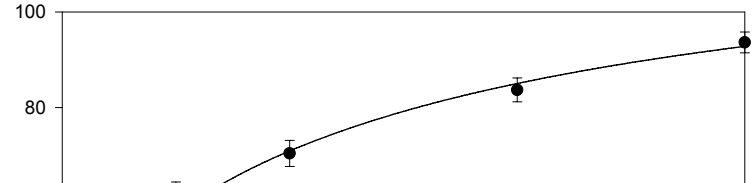
Figure S4: the percentile release of C_2-ceramide versus time for the DPS-C2 (●) and the sample (○) where the C_2-ceramide molecules were inserted into empty pores of



the calcined silica frame.

: 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.

*M. Vallet-Regí, A. Rámila, R. P. del Real, J. Pérez-Pariente, *Chem. Mater.* **2001**, *13*, 308.

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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.

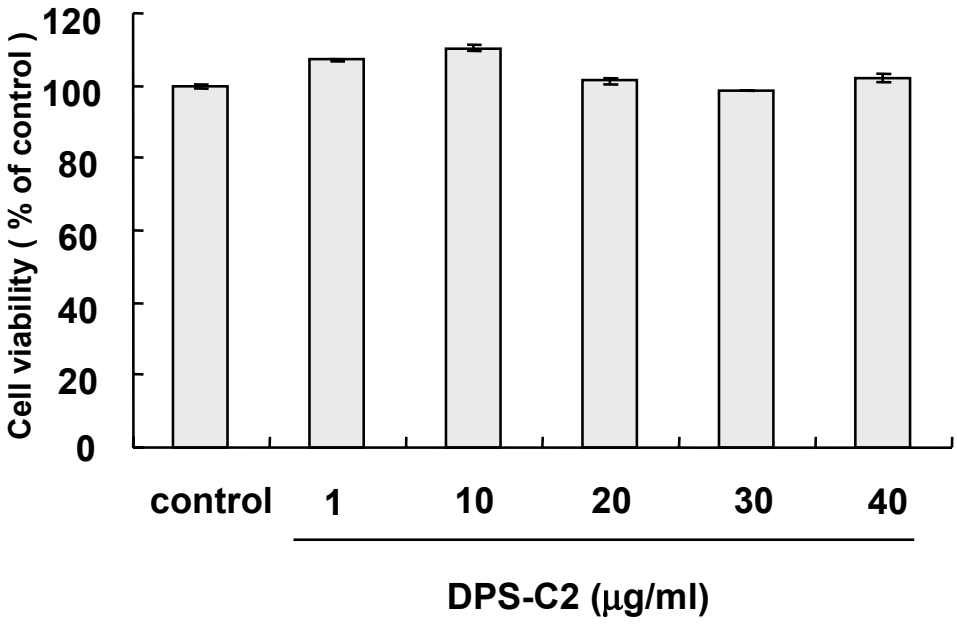

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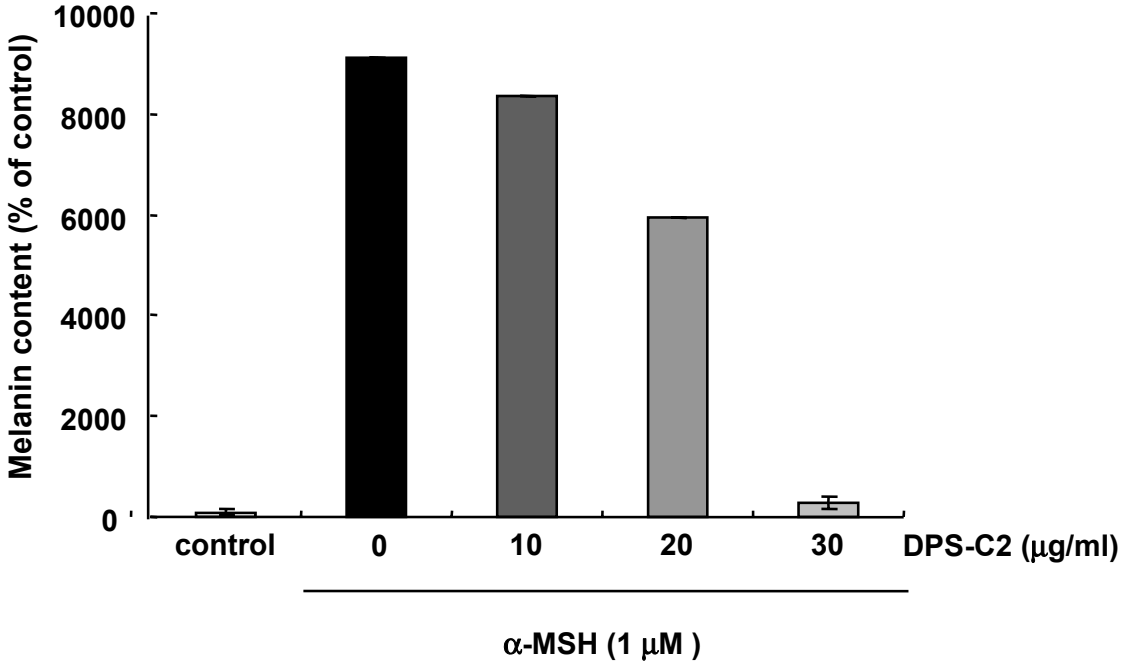
Figure S5: Effects of DPS-C2 on melanogenesis in B16 cells. In the presence of α-MSH (1

μM), the cells were cultured with 10-30 μg/mL of DPS-C2 for 3 day



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