One-pot synthetic route to polymer–silica assembled capsule encased with nonionic drug molecule†

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Received (in Cambridge, UK) 13th February 2007, Accepted 21st May 2007
First published as an Advance Article on the web 8th June 2007
DOI: 10.1039/b702288c

A novel combinational drug delivery system, in which drug molecules could be dually encapsulated by soft (polymer) and hard (inorganic) vehicles has been successfully prepared *via* a simple one-pot synthesis; its improved chemotherapeutic efficacy has been verified through *in vitro* experiments.

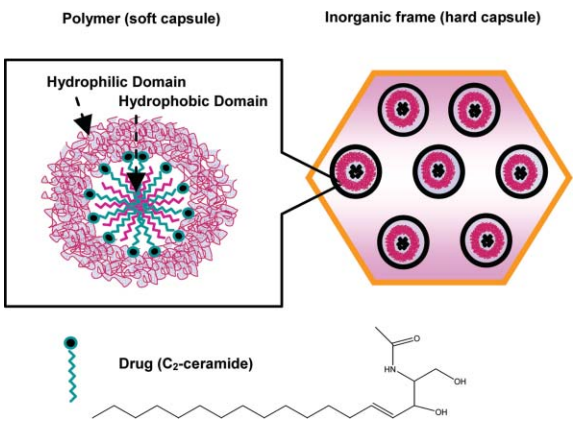
The discovery of new medical agents has attracted a great attention in the past couple of decades. However, despite their high potency, the bolus administration of the drugs may still exhibit low stability and solubility caused by poor chemotherapeutic efficiency. To resolve such problems, drug delivery systems (DDS) have been already a focus in medical science.[1–3]

Among the various DDS, inorganic vectors have been suggested as potential drug reservoirs or delivery vehicles due to stability and hydrophilicity, resulting in more efficient encapsulation and delivery of the drug molecules or DNAs.[4–6]

Recently, mesoporous materials, such as MCM-41 and SBA-15, have been widely investigated as potential inorganic DDS. Due to their adjustable pore diameters and large surface area with abundant Si–OH bonds on the pore surface, these could realize facile adsorption–desorption of drug molecules[7] and even achieve sustained drug release which could improve pharmaceutical kinetics (PK).[8–10] The modification of the channels with chemically removable capping molecules enables a stimuli-responsive pore-opening of MCM-41, whereby photochemical control on the pores, and the therapeutic efficacy of the encased drug molecules could be improved significantly.[11,12] However, there still remain several drawbacks due to the complicated preparation steps; (i) as-synthesized mesoporous materials must be prepared through assembly of organic templates such as alkyl amines with inorganic precursors. (ii) Organic templates should be removed to make valid pore space to load drug by calcination or extraction. (iii) Drug is charged into the mesoporous matrix under dissolving hydrophobic solvent such as hexane. Such factors may result in consuming a lot of cost and time in large scale production. In addition, when loading poorly soluble drugs into mesoporous silica, toxic hexane is used in dissolving drug molecules. In this regard, more simple and biocompatible methods have been demanded for conventional usage.

Herein, to overcome drawbacks of mesoporous materials as a DDS, we propose a combinational drug delivery system, a drug-polymer silica (DPS)-DDS, in which a polymer–drug hybrid is directly encased by inorganic precursors as described (Scheme 1).

DPS-DDS could be directly synthesized by incorporating tetraethyl orthosilicate (TEOS) and template of mesoporous structure in which C_2-ceramide, a water-insoluble model drug,[13] and Pluronic F127 was blended at the same time. Pluronic F127 is a nonionic surfactant with a structural formula of $EO_{106}PO_{70}EO_{106}$, where EO and PO represent hydrophilic ethylene oxide block and hydrophobic propylene oxide block, respectively. Therefore, only Pluronic F127 could play a role as the template for ordered mesoporous silica due to its function as a structure directing agent (SDA).[14] Also, simultaneous assembling of Pluronic F127 and a non-ionic additive could create a new-template structure.[15] In addition, its water-dispersibility and good cellular uptake efficiency made polymeric DDS improve the chemotherapeutic effect of drugs.[16] Due to these features of Pluronic F127, non-ionic drug molecules could be dually encased by soft (polymer) and hard (inorganic) capsules *via* simple one-pot synthesis which is desirable for an economic and biocompatible method. Moreover, DPS-DDS could expect to enhance cellular uptake due to its polymer–drug hybrid structure as well as reproducibility of release by its mesopore structure.



Scheme 1 The schematic illustration of DPS-DDS.

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† Electronic supplementary information (ESI) available: Syntheses, experimental detail, IR, TG-DTA spectra, and additional *in vitro* data. See DOI: 10.1039/b702288c

To prepare DPS-DDS, TEOS was added dropwise into an acidic solution containing both Pluronic F127 and C_2-ceramide. Such a simple step results in a white precipitate, which was then filtered off, washed, and dried in vacuum. Thus obtained sample was denoted as DPS-C2. We also prepared the sample without C_2-ceramide following the same synthetic procedure of DPS-C2 for comparison. The latter was denoted as DPS-AS.

The small angle X-ray scattering (SAXS) pattern of DPS-AS (Fig. 1(a)) prepared in the presence of Pluronic F127 shows typical peaks between 0.6 and 1.5°. The peaks can be indexed as (110), (200), and (211), which represent the cubic ordered mesoporous structure ($Im\bar{3}m$) as previously verified.[14] However, the overall SAXS feature of DPS-C2, although it was almost identical to that of DPS-AS, showed that DPS-C2 was less ordered than DPS-AS in terms of the pore structure. Since the peaks at (200) and (221) were not distinct for DPS-C2 (Fig. 1(b)), the long range order of mesopores in the silica frame-work seemed to be not pronounced.

Such results indicated that the mesoporous structure could be partially perturbed due to the conformational changes of co-template. Even after calcination of the template composed with drug and polymer at 550 °C (Fig. 1(c) and (d)), the inorganic frameworks were maintained without any thermal decomposition.

Because DPS-DDS is mostly employed as powders, its usefulness can also depend on the size, morphology, and pore structure of the particles. As shown in the SEM images (Fig. 2(a) and (b) top), the particles of both DPS-C2 and DPS-AS were spherical with size of about 1 μm. However, according to the TEM images (Fig. 2(a) and (b) bottom), a well-ordered domain of 3D cubic mesostructure along the [111] direction could be seen for DPS-AS while the less ordered structure was observed with DPS-C2. C_2-ceramide, blended with nonionic Pluronic F127, was encased in the inorganic framework, which seemed to change the structural aspect of DPS-C2. This result was quite similar to the binary template system, Pluronic F127 with decane, which led to the formation of a film layer with a negative spontaneous

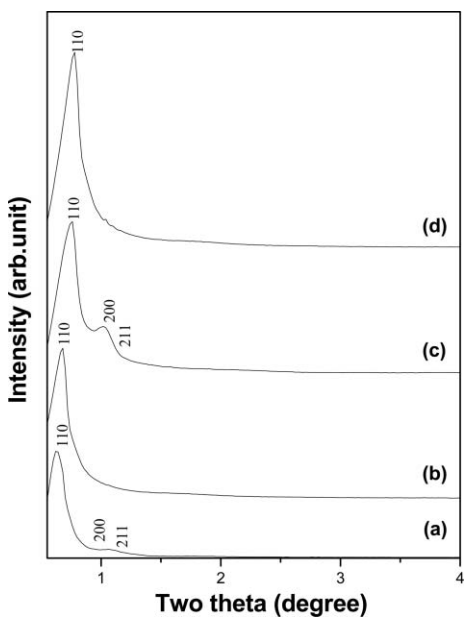

Fig. 1 SAXS patterns for (a) DPS-AS, (b) DPS-C2, (c) calcinced DPS-AS, and (d) calcined DPS-C2.

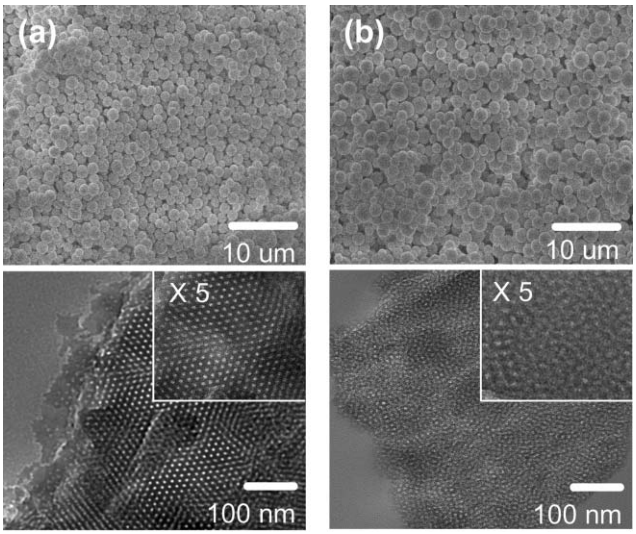

Fig. 2 SEM (top) and TEM (bottom) images of (a) DPS-AS, (b) DPS-C2.

curvature and induced a transition from a cubic liquid crystalline phase to a hexagonal phase.[17] The results from SAXS and TEM for both samples before and after calcination did not show a significant structural difference but a slight decrease in *d* spacing.

As represented in Table 1, the C_2-ceramide content for DPS-C2 was determined to be 9.7 wt% by HPLC analysis with an evaporative light-scattering detector (ELSD). From the total organic content (37.2 wt%) analyzed by TG-DTA, Pluronic F127 was estimated to be 27.5 wt%.

In order to apply the DPS-DDS, its biocompatibilty and bioavailability should be understood, since the drug delivery carrier itself sometimes causes undesired side effects such as cytotoxicity, low therapeutic efficacy, *etc.* In this regard, B16 murine melanoma cells (B16 cells) were employed to investigate bioactivity for DPS-C2 since C_2-ceramide is involved in a signalling pathway to activate the extracellular signal-regulated kinase (ERK), which leads to the suppression of tyrosinase activity and melanin synthesis.[18] The B16 cells were exposed to 38 μg ml^{-1} of DPS-C2 in the presence of α-melanocyte stimulating hormone (α-MSH) for 3 days, and then the extracellular melanin production was measured. An addition of α-MSH gave rise to an increase of melanin content in the media, but the DPS-C2 treatment led to a reduction of α-MSH-induced extracellular melanin accumulation in a dose-dependent manner. The effect of DPS-C2 on melanin synthesis was compared to that of C_2-ceramide itself. At the same molar concentration of C_2-ceramide, DPS-C2 showed a substantial suppression of melanin formation, which was approximately three times more efficient than the case with C_2-ceramide itself (Fig. 3). Its activity was stronger than kojic acid which is known as

Table 1 Percentage of molecular content for DPS-AS and DPS-C2 (wt%)

Sample	Water	F127	C_2-ceramide	SiO_2
DPS-AS	2.5	50.1	0	47.4
DPS-C2	4.2	27.5	9.7	59.6

[a] Measurement by TG and HPLC-ELSD.

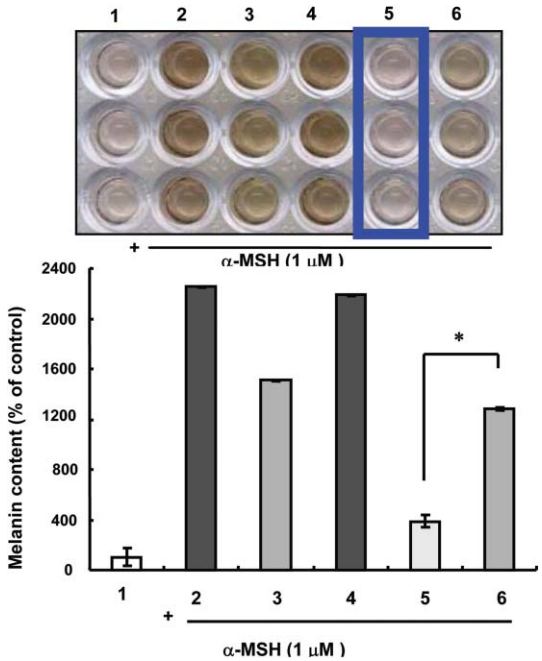

Fig. 3 The qualitative (top) and quantitative (bottom) effects of DPS-C2 compared to C_2-ceramide on melanogenesis in B16 cells. 1, control; 2, α-MSH (1 μM); 3, kojic acid (10 μg ml^{-1}); 4, DPS-AS (38 μg ml^{-1}); 5, DPS-C2 (38 μg ml^{-1}); 6, C_2-ceramide (10 μM). Wells 2–6 in the presence of α-melanocyte stimulating hormone (α-MSH) for 3 days. The results shown are the averages of triplicate experiments ± SD. Statistical significance was evaluated by t-test in the SPSS release 12.0 (SPSS Inc). *P <0.01 compared to the C_2-ceramide (10 μM)-treated group.

a commercial tyrosinase inhibitor. This was due to the enhanced solubility and release property of C_2-ceramide, which resulted from the synergetic effect by DPS-DDS.

In summary, we have demonstrated, for the first time, DPS-DDS to combine both polymer and inorganic molecules as DDS. The simplicity of a one-pot synthesis suggested being beneficial for large-scale production. The therapeutic efficacy of DPS-C2 on melanoma cells was found to be higher than that of C_2-ceramide itself. Therefore, the novel DPS-DDS may have a potential as an advanced drug delivery system.

This research is financially supported by the Korean Ministry of Health and Welfare through a grant of the Korea Health 21 R&D Project (02-PJ1-PG1-CH11-0001). We are grateful to the Korea Science and Engineering Foundation (KOSEF), SRC/ERC program (R11-2005-008-01001-0), and BK21 program.

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