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## COMMUNICATION

## Facile Microcapsule Fabrication by Spray Deposition of a Supramolecular Hydrogel

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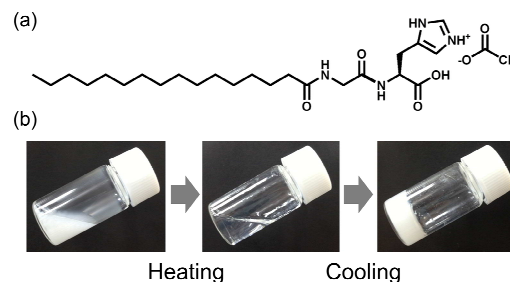
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**A peptide amphiphile dispersed in water induces the formation of a supramolecular hydrogel. By spraying this hydrogel onto a solid surface, its hierarchical fibril structure remained unchanged even after water evaporation. Interestingly, there were microcapsules on the substrate surface as a result of the process of spray-drying the hydrogel.**

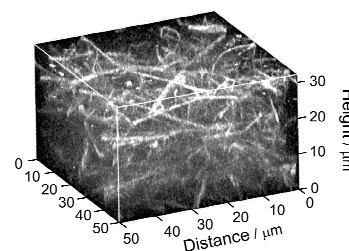
Microcapsules including hollow spheres offer a great potential in various applications such as drug delivery, (bio)catalysis, paper coating, cosmetics and so forth because of their low effective density, high specific surface area and remarkable encapsulation capability.<sup>1,2</sup> Microcapsules are commonly fabricated by coating the surface of colloidal templates with layers of the desired material and then removing the templates by means of calcinations and/or chemical etching.<sup>3</sup> This straightforward approach works well for a variety of materials such as polymers, ceramics, and composites and metals.<sup>4</sup> However, such approach involves multi-step processes requiring a careful examination of the experimental condition in each step. Thus, to expand the applicability of the microcapsules, the development of a facile fabrication method is desired.

Supramolecular hydrogels (SMGs) are soft materials capable of immobilizing a substantially large amount of water within the three-dimensional network of the fibrils formed by the self-assembly of low molecular-weight molecules. To form the hydrogel, a peptide amphiphile, *N*-palmitoyl-Gly-His trifluoroacetate (PalGH), was dispersed in water. In this study, we demonstrate a one-step fabrication of microcapsules by a conventional spray technique using this SMG. When this is sprayed onto a substrate, the resultant film, after drying off the water, contains microcapsules composed of the three-dimensional fibril network of the amphiphilic molecules. To the best of our knowledge, this is the first demonstration of a microcapsule preparation by simply spraying SMGs on a surface.

PalGH was synthesized according to the previously-reported method.<sup>5</sup> Panel (a) of Fig. 1 provides the chemical structure of



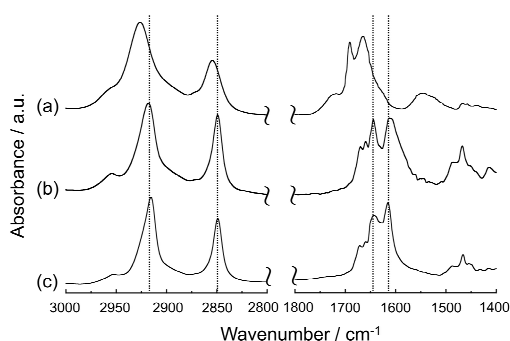
**Fig. 1** (a) Chemical structure of the hydrogelator, PalGH and (b) photographic images of the PalGH-water mixture during the preparation of the hydrogel. [PalGH] = 0.5 wt%



**Fig. 2** CLSM image of the hydrogel containing 8-anilino-1-naphthalenesulfonate (ANS) as a fluorescence dye. [PalGH] = 0.5 wt% and [ANS] = 60 μM.

PalGH, while panel (b) shows the PalGH and water in various physical states. An aqueous dispersion of PalGH was heated at 363 K for 10 min, leading to a clear solution. Cooling this solution to room temperature and then aging for 1 hour gave a hydrogel, which did not flow even after tilting the vial containing it at an angle.

Fig. 2 shows the three-dimensional image obtained by confocal laser scanning microscopy (CLSM). As a fluorescence probe, 8-anilino-1-naphthalenesulfonate (ANS) was used. ANS dye exhibits a much stronger emission when in a hydrophobic environment compared to that in a hydrophilic one, making it possible to stain a



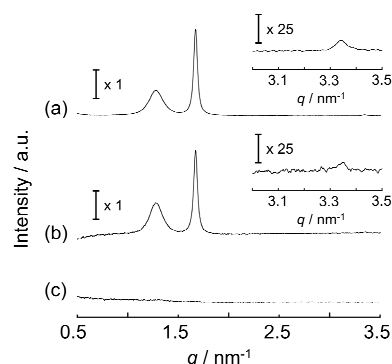
**Fig. 3** FT-IR spectra for PalGH in (a) the DMSO solution, (b) the gel, and (c) in the film obtained after the spray-dry process.

hydrophobic core in a micelle-like assembly.<sup>6</sup> This image reveals that a three-dimensional network of the fibrils was formed in the gel. Assuming that the network structure produces stronger mechanical properties, it can be claimed that the gel formation results from the network due to the entanglement of the fibrils formed by the self-assembly of the PalGH molecules.

The spray deposition of the gel was made using a conventional spray vial (Maruemu Co., Japan) having a push-type trigger. The gel was sprayed on a silicon substrate having a native oxide layer in a perpendicular direction to the substrate surface. The distance between the spray nozzle and the substrate was kept to be 30 mm. During the spray process, the gel was broken and transformed into a fluid state, namely a sol.<sup>7</sup> The resultant sol spread on the substrate surface without any dripping. Water in the sol on the substrate was removed by pre-drying under an ambient atmosphere for 6 hours and then further dried off under a vacuum for 24 hours.

We examined the aggregation states of the gel and the spray-deposited film by Fourier-transform infrared (FT-IR) spectroscopy and small angle X-ray scattering (SAXS) measurements. Fig. 3 shows the FT-IR spectra of PalGH in (a) dimethyl sulfoxide (DMSO), (b) the initial gel and (c) the film after the spray-dry process, respectively. Deuterated solvents were used for the preparation of all samples. The spectra for PalGH in the DMSO solution and the gel were obtained after removing the contribution from the solvent.

In the gel state, absorption peaks, which are assignable to the CH<sub>2</sub> symmetric and antisymmetric stretching vibrations ( $\nu_{s,CH_2}$  and  $\nu_{as,CH_2}$ ), appeared at 2849 and 2918 cm<sup>-1</sup>. These values were smaller than those obtained for the DMSO solution (2855 and 2926 cm<sup>-1</sup>) where the PalGH molecules were homogeneously dispersed. This indicates that the alkyl chain in PalGH was extended with a higher population of the *trans* conformation in the gel state. Also, the gel exhibited peaks due to the C=O stretching vibration in the amide moiety ( $\nu_{C=O}$ ) at two different wavenumbers, 1644 and 1612 cm<sup>-1</sup>. Two other peaks at 1659 and 1670 cm<sup>-1</sup>, which were assignable to the vibration of the C=O group in the carboxyl group ( $\nu_{COOH}$ ) and trifluoroacetate ( $\nu_{TFA}$ ), respectively,<sup>8</sup> can be observed. On the other hand, the DMSO solution provided the  $\nu_{C=O}$  peaks at 1664 cm<sup>-1</sup>, which also included in part the  $\nu_{COOH}$  peak, and the  $\nu_{TFA}$  peaks at 1691 cm<sup>-1</sup>. The lower position of the  $\nu_{C=O}$  peaks observed for the gel relative to that for the DMSO solution are characteristic of hydrogen bonding.<sup>9</sup> Thus, it is most likely that the two  $\nu_{C=O}$  peaks observed



**Fig. 4** SAXS profiles of PalGH assembly in (a) the gel, (b) the sol obtained by spray deposition and (c) the film obtained after the spray-dry process.

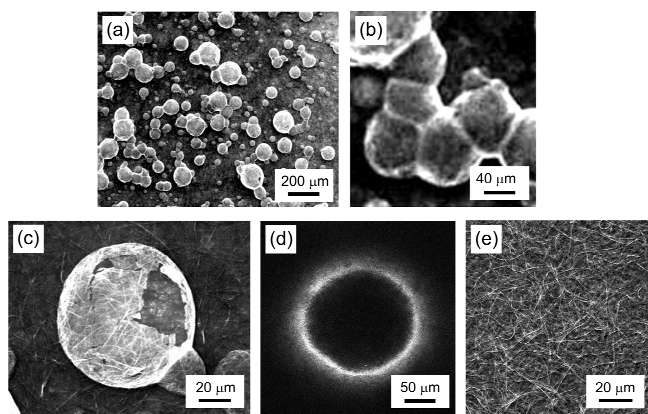
for the gel are assignable to the C=O groups, which form weak and strong hydrogen bonding interactions, respectively.

Here, it is noteworthy that the peak positions of the  $\nu_{s,CH_2}$ ,  $\nu_{as,CH_2}$  and  $\nu_{C=O}$  peaks observed for the film were comparable to those for the gel. This observation implies that the PalGH molecules form an assembly, accompanied by alkyl chain ordering and hydrogen bonding via C=O groups in the gel, and the assembled state remained unchanged even after the drying process.

Fig. 4 shows SAXS profiles for (a) the initial gel, (b) the sol obtained after the spray deposition and (c) the film obtained after the spray-dry process, respectively. The gel has a broad scattering peak at a scattering vector ( $q$ ) of 1.3 nm<sup>-1</sup>, corresponding to a domain spacing ( $d$ -spacing) of 4.9 nm. The length of the PalGH molecule is approximately 3.3 nm. The  $d$ -spacing observed by SAXS was larger than the molecular length but shorter than twice the molecular length. Invoking that the PalGH molecules form an assembly with interdigitated alkyl chains, it seems reasonable to imply that the assembly possesses a fibrous morphology similar to that observed for other peptide-based amphiphiles.<sup>6d,10</sup> In addition, sharp peaks were observed at  $q = 1.7$  and 3.3 nm<sup>-1</sup>, corresponding to  $d$ -spacings of 3.8 and 1.9 nm, respectively. The peaks were detected at characteristic positions in a ratio of 1:2. The scattering peaks at  $q$  ratios of 1:2:3:4 etc. are characteristic of a lamellar system.<sup>11</sup> Thus, it is conceivable that the PalGH molecules also form a lamellar-like assembly. Both fibrous and lamellar-like assemblies were much smaller than the fibrils observed by CLSM. If that is the case, the fibrous and lamellar-like assemblies co-aggregate with each other in the gel, producing fibrils with a size in the micrometer range.

In the case of the sol obtained by spray deposition, the SAXS profile was almost the same as that observed for the gel. This indicates that the spraying process does not affect the original assembled state even during the gel-to-sol transition. After being dried, however, the scattering peaks disappeared. Taking into account the FT-IR result that the molecular assembled state was preserved even after being dried, it is likely that the assemblies do not form any long-range periodic structures.

Panel (a) of Fig. 5 shows scanning electron microscopic (SEM) images for the film obtained after the spray-dry process. Micro-sized particles were observed while some particles were fused to each other, as shown in Fig. 5(b). The diameter of the particles is in

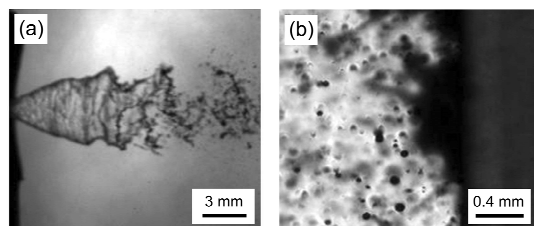


**Fig. 5** (a-c and e) SEM images and (d) CLSM image (at the confocal plane) of the film obtained by (a-d) the spray deposition and (e) the casting method. [ANS] = 60  $\mu\text{M}$  for the panel (d).

the range of 20 - 150  $\mu\text{m}$  with an average value of 55  $\mu\text{m}$  (See Fig. S1 in ESI†). Panel (c) of Fig. 5 is a part of the image in panel (a) at a higher magnification. Interestingly, the particles with a diameter of approximately 1  $\mu\text{m}$  possess a hollow morphology formed from the aggregation of the fibrils. Such a hollow morphology was also confirmed by CLSM as shown in Fig. 5(d). On the other hand, when the gel was cast on the substrate, the microcapsules, as shown in panels (a-d), were not observed at all. Instead, a flat film was formed, as shown in Fig. 5(e). Thus, it is apparent that the spray-dry process causes the formation of the microcapsules.

To discuss the origin of the microcapsule formation, the spraying behavior was observed by a high-speed camera attached with a magnifying lens. Panel (a) of Fig. 6 shows a snapshot obtained near the spray nozzle during the spray process of the gel. The atomization features wavy-sheet formation in the direction perpendicular to the primary flow, which was often observed for waterborne coatings.<sup>12, 13</sup> This behavior was quite similar to that obtained from spraying just pure water, suggesting that the spray droplets were in the sol state. Panel (b) of Fig. 6 shows an enlarged snapshot observed near the substrate. Droplets with diameters ranging from 100  $\mu\text{m}$  to 800  $\mu\text{m}$  came out from the nozzle at a velocity of approximately 10  $\text{m}\cdot\text{s}^{-1}$ , violently collided on the substrate surface, and then collapsed or bounced back. Thus, although the droplets and microcapsules are comparable in their sizes, they cannot be directly associated with each other.

As a result of the violent collision of the spray droplets on the substrate, air bubbles were generated in the sol. The subsequent evaporation of water from the sol should be accompanied by the fibrils covering the surface of the bubbles, resulting in the hollow



**Fig. 6** Snapshots of the spray deposition for the gel observed (a) near the spray nozzle and (b) the surface of the substrate.

morphology. If that is the case, a cluster of the bubbles, that is, a set of fused microcapsules, would satisfy Plateau's laws that describes that three faces meet at an angle of 120° to minimize the interfacial energy.<sup>14</sup> This is exactly what was observed in Fig. 5(b). The angle between two of the borders in three fused microcapsules was 120°. This might support our claim that the bubbles generated in the sol behave as a template for the microcapsule formation.<sup>15</sup>

## Conclusions

In conclusion, we have characterized a film prepared simply by spray deposition of a hydrogel based on a fibril network formed as the result of self-assembly of low-molecular-weight molecules. The film so obtained contained the microcapsules. Casting the gel instead of spraying it did not produce microcapsules. The droplets obtained from spraying the gel intensely collided on the substrate surface, leading to their collapse and splash-back. Such events might generate air bubbles that can act as templates for the formation of the microcapsules. Here, it should be emphasized that the microcapsules were produced under an ambient condition by a simple spray-dry process. The finding of this study provides useful concept for the fabrication of microcapsules. Also, the spray-deposited film with the microcapsules possesses a potential as a drug carrier, cell scaffold and so forth. In fact, as demonstrated by CLSM, functional molecules can be loaded on the microcapsules.

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## Notes and references

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- 15 If the air bubbles are conducted into the casting solution, the microcapsules with various sizes are formed. This clearly makes it

clear that the air bubbles act as a template for the microcapsule formation.

## Table of Contents

When a supramolecular hydrogel, that was based on the self-assembly of low-molecular-weight molecules, was sprayed onto a solid substrate, the resultant residue after drying off the water contained microcapsules composed of the fibrils.

