Photo-responsive gel droplet as a nano- or pico-litre container comprising a supramolecular hydrogel†

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Photo-responsive gel droplets having nano- or pico-L volume that showed photo-induced gel–sol transition were successfully developed, for which the inter-droplet mass transport and the subsequent enzymatic reactions in the interior of the gel droplets were photo-triggered.

Photo-responsive hydrogels are regarded as one of the intriguing biomaterials applicable to biological and medical fields. This is because light is one of the ideal stimuli for fabricating and manipulating various properties of semi-wet materials due to its clean, fast, and spatio-and-temporarily controllable features. However, hydrogels having an efficient photo-responsive property have not been sufficiently developed,[1,2] compared to other types of hydrogels responsive to physical stimuli such as pH or heat. It is now crucial to explore the capability and the limitations of photo-responsive hydrogels as potential biomaterials.[3]

Supramolecular hydrogels consisting of self-assembled small molecules have recently emerged and are expected to be novel soft materials with the benefit that elaborate design of their component small molecules may directly influence their macroscopic hydrogel properties.[4] We recently found that a supramolecular hydrogel **1** bearing fumaric amide as a *trans–cis* photo-switching module shows gel–sol transition induced by UV light (Chart 1).[5] The *trans–cis* photo-isomerization of the double bond of **1** effectively caused the formation or deformation of the self-assembled supramolecular fibers, so as to yield the macroscopic hydrogel or the corresponding sol, respectively. Here, we report that a photo-responsive supramolecular gel droplet with nano- or pico-L volume comprising **1** was prepared in hexadecane. The inter-droplet mass transport and the subsequent enzymatic reactions confined in the interior of the gel droplets were effectively photo-controlled.

By injecting 2 μL of the hot sol of **1** in water into hexadecane at room temperature followed by vortex mixing, spherical sol droplets are formed which gradually turn into gel droplets (*ca.* 50–100 μm in diameter, that is 60 pL–0.5 nL in volume) by self-assembly (Fig. S1†, Fig. 1a and b). Unlike the limp droplets of the sol or water, the gel droplets seem relatively stiff. The surface morphology of the gel droplet was uneven, in contrast to the smooth surface of the sol droplet. The 3D-mapping of a supramolecular gel droplet stained with octadecyl-rhodamine (C_{18}-Rh) by confocal laser scanning microscopy (CLSM) revealed well-developed fibrous networks of the self-assembled gelator **1** within the droplet sphere as shown in Fig. 1b (Movie S1†). We also found a density gradient of the

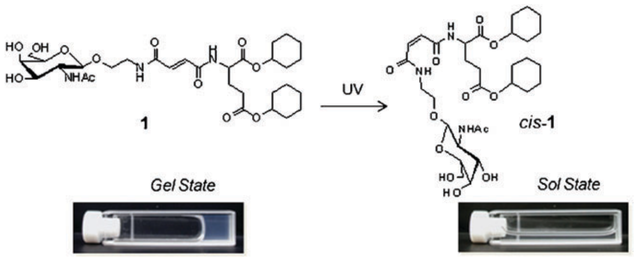

Chart 1

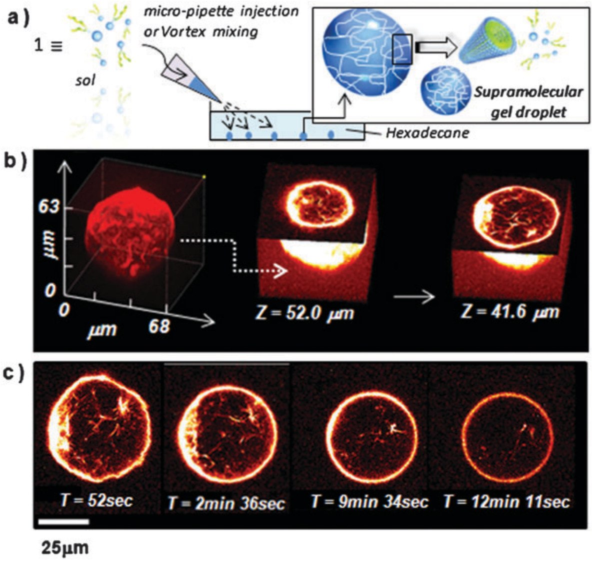

Fig. 1 CLSM images of the supramolecular gel (**1**) droplet in hexadecane. (a) Schematic illustration of preparation of supramolecular gel droplets. (b) 3D-fluorescence image of hydrogel **1** droplet (*ca.* 60 pL) stained with a hydrophobic rhodamine ([C_{18}-Rho] = 100 μM, excitation wavelength 543 nm) and the *Z*-axial profiles of the gel droplet (Movie S1†). (c) The inner space images of the gel (**1**) droplet during UV irradiation (Movie S2†) with a low-pressure mercury vapor lamp.

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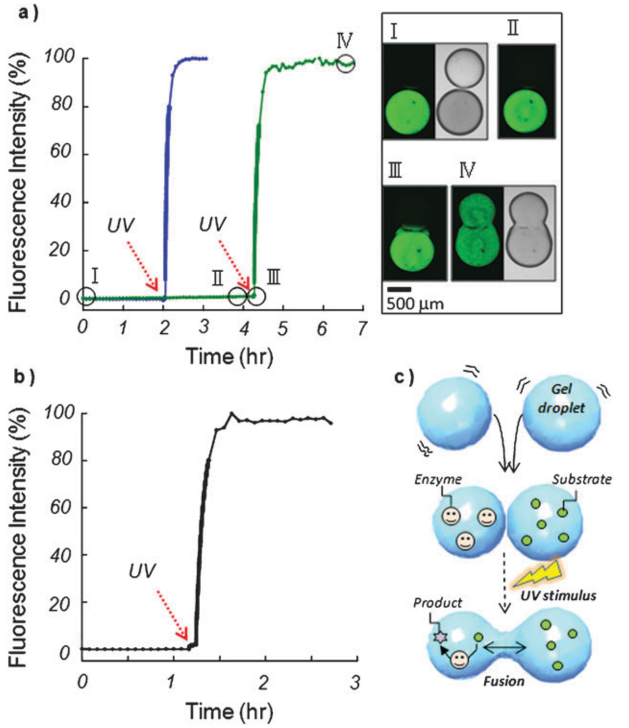

Fig. 2 Photo-triggered fusion of two gel droplets. (a) Time courses of the transport (fluorescence intensity) (%) of Cy-5 (blue) and fluorescein (green) from the dye containing gel droplet to the blank one. Red arrows indicate the initial time of UV light (*ca.* 2–3 min) irradiation with a low-pressure mercury vapor lamp. Inset: fluorescence and DIC images of the gel droplets at I, II, III and IV stage in the panel a. (b) Enzymatic reaction triggered by the photo-fused gel droplets containing enzyme (AP, the left droplet) and the substrate (the right droplet). Time course of the fluorescence intensity of a region in the AP-containing droplet. (c) Schematic illustration of regulation of enzymatic reaction using photo-triggered partial fusion of two gel droplets.

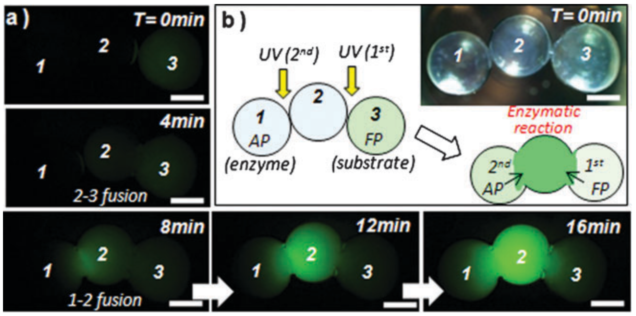

Fig. 3 The photo-induced stepwise fusion between three gel droplets using laser light (266 nm) for *ca.* 2 s. (a) Fluorescence images of the three distinct gel droplets in contact containing different interiors, that is AP in droplet 1, none in droplet 2, and the substrate in droplet 3. The focused laser light was irradiated step-by-step to the interface between droplets 2 and 3, and subsequently between droplets 1 and 2. (b) Schematic illustration of this experiment. Inset: bright field images of the three droplets. Scale bar: 500 µm.

gel fibers, with the fibers in the outer layer much denser than in the inner space. UV irradiation of the gel droplet changed the rough surface of the droplet into a smooth spherical surface, with concurrent disappearance of the network of inner fibers, corresponding to the photo-induced gel-to-sol transition of droplets (Fig. 1c and Movie S2†). The gel state of the interior space of the droplet was probed by the Brownian motion of nano-beads. As shown in Fig. S2†, we observed by CLSM that the Brownian motion of all beads (250 nm) embedded in the gel droplet stopped, indicating that the formation of the gel fiber network entrapped the nano-beads in the inner space. The Brownian motion of the nano-beads re-started when the sol droplet was photo-produced.

Because of their stiffness, two gel droplets brought into contact with each other retain their shape, whereas two sol or water droplets readily fuse into one larger droplet. Interestingly, two gel droplets can be partially fused into a dumbbell shape by the stimulus of light (UV irradiation) as shown in the inset of Fig. 2a. We observed by CLSM that short-time (2–3 min) UV irradiation caused closer contact and subsequent partial fusion of two gel droplets.[6] The mass transport between two gel droplets in contact was examined using fluorescent probes such as water-soluble fluorescein (Fl) and Cy-5. When a gel droplet that did not contain Fl was placed in contact with another gel droplet containing Fl, no transport of Fl occurred within 4 h as shown in Fig. 2a, indicating entrapment of the dye in the gel droplet. In contrast, when two gel droplets were partially fused by UV irradiation into the dumbbell shaped droplet, Fl rapidly diffused into the other space until equilibration (within 20 min). This implies that the mass transport between two distinct gel spaces was successfully photo-regulated.

Besides simple diffusion, enzymatic reactions in the gel droplet could be photo-regulated. We previously demonstrated that our supramolecular hydrogels can provide a suitable matrix to immobilize native proteins and enzymes without denaturation.[7] Even though two distinct gel droplets containing alkaline phosphatase (AP, a hydrolytic enzyme) or the corresponding fluorogenic substrate were placed in contact, the enzymatic reaction did not take place (Fig. 2b). Upon the photo-induced partial fusion of the two droplets, the strong fluorescence emission of the AP-catalyzed reaction product rapidly appeared at the interface, indicating that the reaction had been initiated (Fig. 2c). A similar photo-triggered reaction was also carried out for glucosidase (Fig. S4†). Using focused laser light, more precise photo-fusion between gel droplets was carried out. Fig. 3 shows three distinct gel droplets in contact, with different contents, that is AP in droplet 1, none in droplet 2, and the substrate in droplet 3. When the interface between droplets 2 and 3 was irradiated (for 2–3 s) with laser light (266 nm), 4 min after mechanical contact between droplets, very weak fluorescence was diffused from droplet 3 to droplet 2, but not droplet 1. This indicates that the photo-fusion area was strictly limited to the interface between droplets 2 and 3. Subsequently, more than 4 min later, the interface between droplets 1 and 2 was photo-irradiated by the laser to fuse the two droplets. Immediately after the fusion, strong emission appeared at droplet 2, suggesting that the AP-catalyzed hydrolysis took place mainly in the central droplet 2. This result indicated that spatial and temporal control of the mass transport and the consequent chemical reactions in the confined space in the gel droplet are accomplished by use of photo-responsive gel droplets.[8]

In conclusion, a photo-responsive gel droplet having nano- or pico-L volume was successfully developed using a supramolecular hydrogelator that induced the gel–sol transition by UV light.[2,9] Using the present system, mass transport between gel droplets was controlled at will by light, and this may be applicable to intelligent delivery systems of various substrates of nano–micrometre range such as nanoparticles or cells as a nano- or pico-L volume container.

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