Appendix A. Supplementary data

Multicompartmental microcapsules for enzymatic cascade reaction by gas-shearing method with surface gelation

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1. Experimental Section

1.1 Cell culture

L929 cells were cultured in DMEM with 10% FBS. The cell culture flasks were maintained in a humidified incubator at 37 °C in a 5% CO_2 environment. The cell culture medium was replaced every 3 days.

1.2 Cell viability by live/dead staining

For measuring cell viability through live/dead staining, cells were seeded in 12-well plates at a cell density of 1×10^5 cells per well for 24 h. Cell culture media containing 50 Janus microspheres or microcapsules were applied to the cells for 24 h, 48 h, or 72 h. The medium was removed, and the cells were washed three times with PBS. Finally, the cell viability rates were determined based on the green and red fluorescence of calcein-AM and red PI, respectively (10 min staining). Cells were imaged using a fluorescence microscope (IX 53, OLYMPUS, Japan). Triplicates were used for each group.

1.3 Cell viability by MTT assay

The cells were seeded in 96-well plates at a cell density of 6000 cells per well for 24 h. Cell culture media containing 10 microparticles were added to the cells and incubated for 24 h, 48 h, or 72 h. Subsequently, 20 μL 5 mg/mL MTT was added to each well, and the cells were incubated at 37 °C for 4 h. The medium was then carefully removed, and 150 μL DMSO was added into each well for 20 min to dissolve the formazan crystals. The absorbance of each well was measured with a microplate reader at 492 nm (BIO-TEK, EL800). The experiment was conducted in quintuplicate, and the results were shown as mean ± SD.

1.4 Hemolytic activity test

Erythrocytes were separated from the mouse blood via centrifugation at 1000 rpm for

10 minutes. The obtained erythrocytes were washed three times with PBS and then diluted in PBS to achieve a final concentration of 10% (v/v). A volume of 500 μL PBS containing 25 microparticles was added to 500 μL erythrocytes stock, then shaken in an incubator at 37 °C for 1 h. Triton x-100 at the concentration of 0.1% (v/v) was used as the positive control, and PBS was used as the negative control. The mixtures were then centrifuged at 8000 rpm for 1 min. The OD value of the supernatant was quantified at 540 nm using a microplate reader. Each absorbance data point was obtained by measuring the samples, and the deviations of the three tests were also determined. The hemolytic ratios of the samples were calculated as follows:

$$\text{Hemolytic ratio (\%)} = (D_t - D_{nc}) / (D_{pc} - D_{nc}) \times 100$$

where D_t represents the OD value of tested sample, and D_{nc} and D_{pc} denote the OD values of the negative and positive controls, respectively.

2. Table & Figures

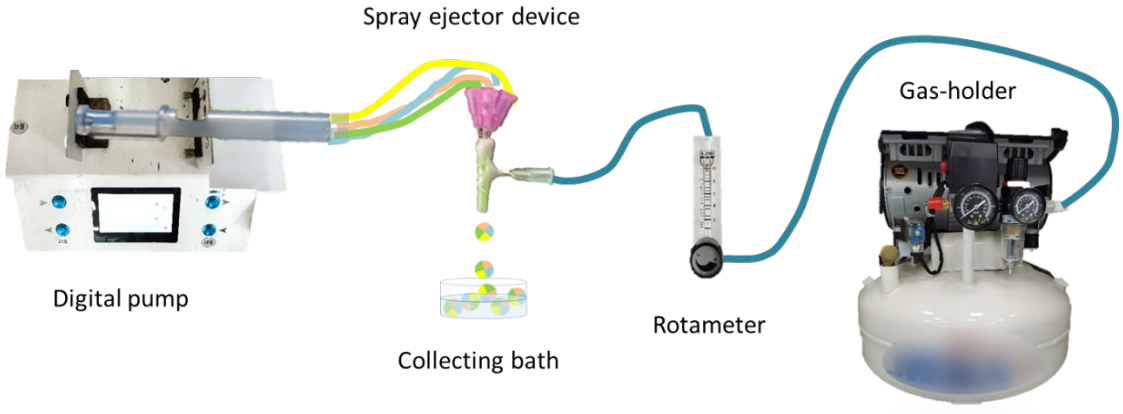
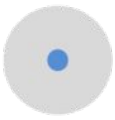
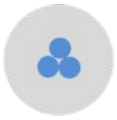
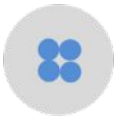
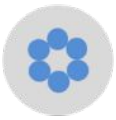


Figure S1. Equipment used to generate microparticles through gas-shearing method.

Table S1. Images of one-, two-, three-, four-, six- or eight-faced needles to fabricate corresponding multicompartmental particles.

	A	B	C	D	E	F
Needle type	One-face	Two-face	Three-face	Four-face	Six-face	Eight-face
Image						
Bottom view						

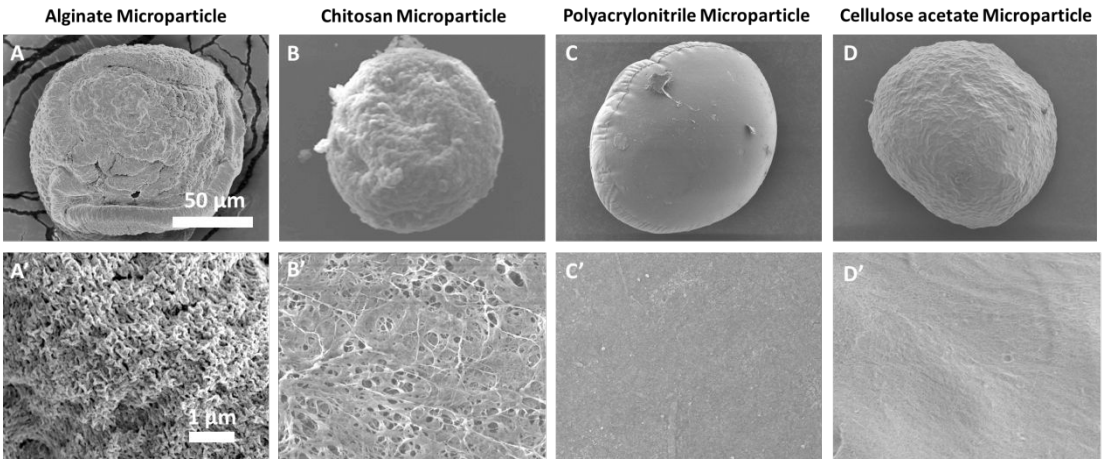


Figure S2. SEM images of alginate (A,A'), chitosan (B,B'), polyacrylonitrile (C,C') and cellulose acetate (D,D') microparticles.

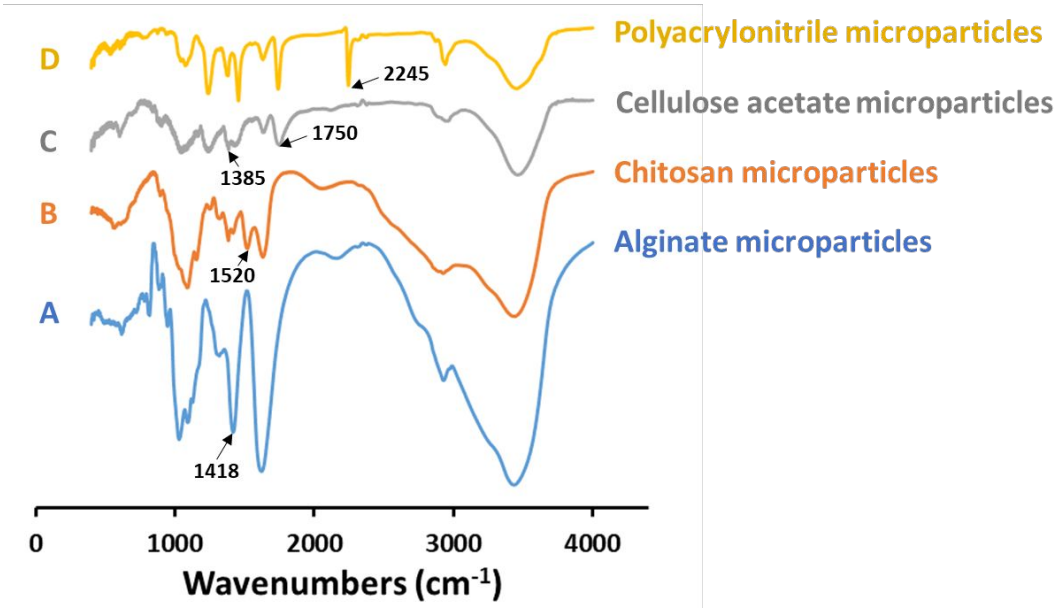


Figure S3. FTIR spectra of alginate (A), chitosan (B), cellulose acetate (C) and polyacrylonitrile (D) microparticles. There is a characteristic adsorption peak at 1418 cm^{-1} attributed to the symmetric $–COO^-$ stretch of alginate acid (Figure S3A). Figure S3B exhibited a characteristic absorption peak at 1520 cm^{-1} due to the $–NH_2$ groups in the chitosan structure. The characteristic bands of cellulose acetate microparticles at 1385 and 1750 cm^{-1} were due to the stretching of $–C–CH_3$ and the carbonyl (C=O) from acetyl groups, respectively (Figure S3C). A prominent adsorption peak at 2245 cm^{-1} attributed to the –C≡N group from polyacrylonitrile was observed in Figure S3D.

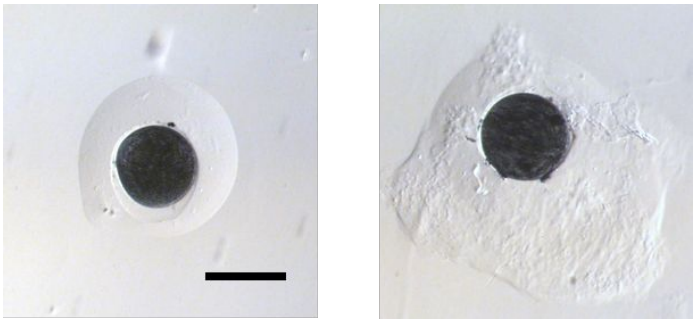


Figure S4. Optical micrographs of microcapsules with an incubation time of 120 s (left) and microcapsules incubated in alginate solution with a GDL concentration of 10 mg/mL (right). The scale bars are 200 μm.

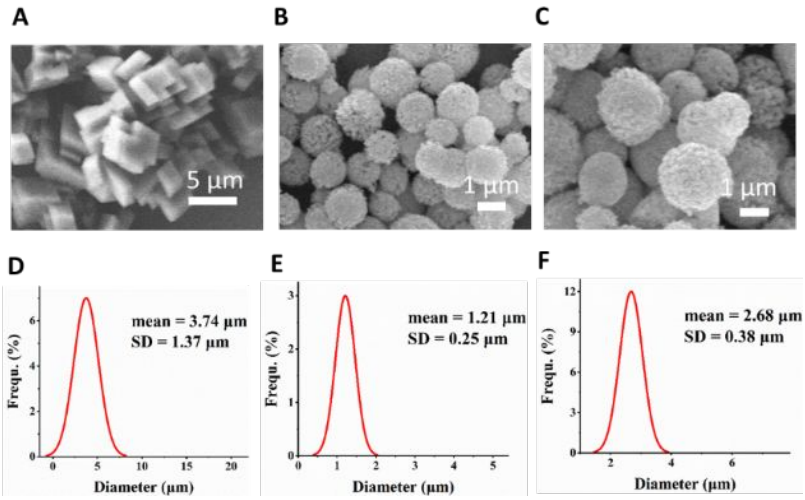


Figure S5. SEM images and crystallite size distributions of calcite (A,D), vaterite1 (B,E) and vaterite2 (C,F).

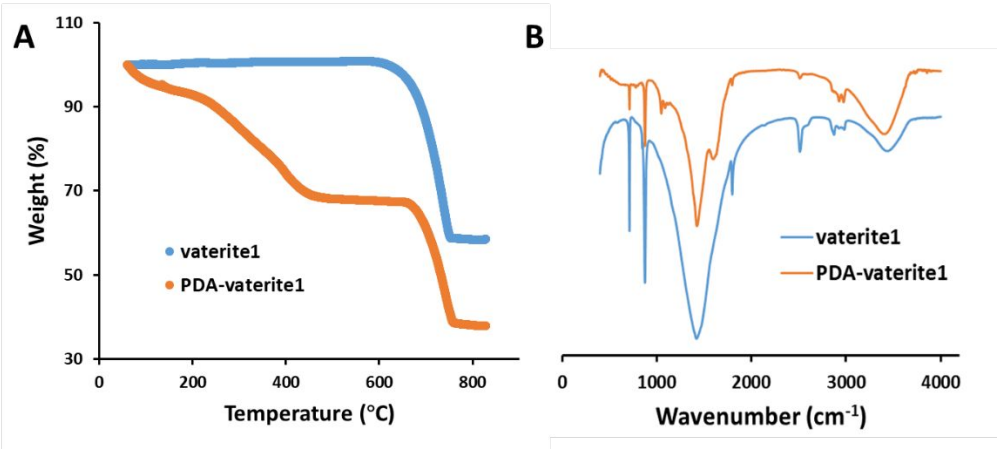


Figure S6. TG (A) and FTIR spectra (B) of vaterite1 and PDA-vaterite1.

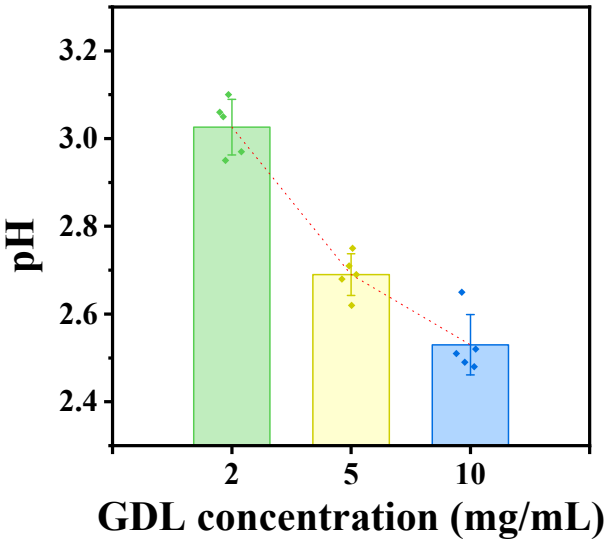


Figure S7. pH values of different concentrations of GDL in equilibrium state.

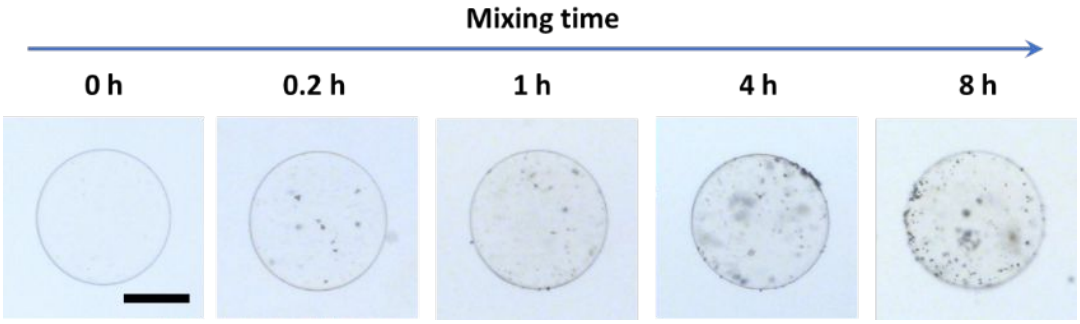
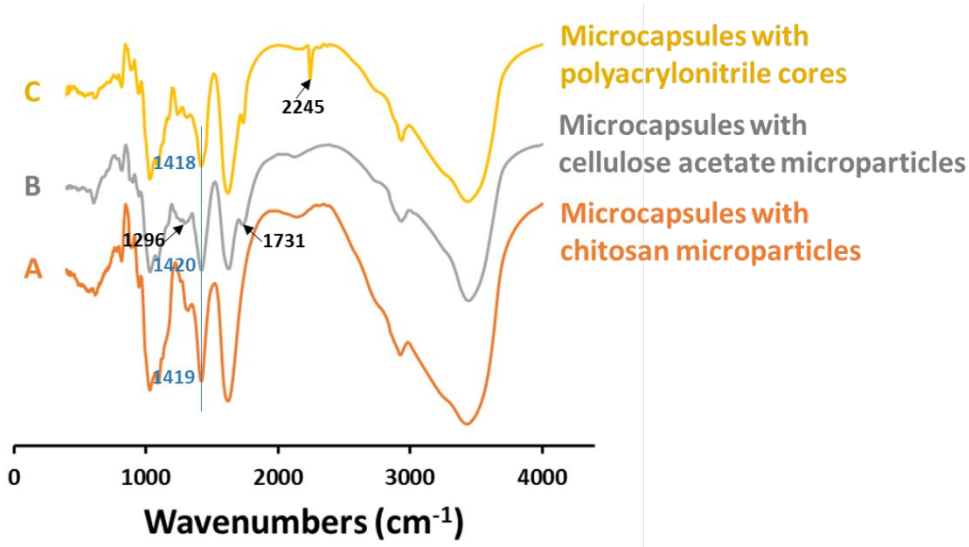


Figure S8. The gradual increase of PDA-CaP attached to the surface of core particles with the prolongation of mixing time. The scale bar is 100 μm.



131

132 Figure S9. FTIR spectra of alginate microcapsules with chitosan (A), cellulose acetate
133 (B) and polyacrylonitrile (C) cores. The characteristic adsorption peaks at 1418, 1419
134 or 1420 cm^{-1} attributed to the symmetric $–COO^-$ stretch of alginate acid, indicating that
135 all kinds of cores were encapsuled by alginate gels. The characteristic absorption peak
136 at 1296 and 1731 cm^{-1} due to $–C–CH_3$ and –C=O from cellulose acetate, and at 2244
137 cm^{-1} attributed to the –C ≡ N group from polyacrylonitrile were still noticed in
138 microcapsules. Peak of $–NH_2$ was much weaker in spectrum of microcapsules with
139 chitosan cores, probably caused by interaction between amino group from chitosan and
140 carboxyl group from alginate when forming the microcapsules.