Electronic Supplementary Information (ESI)

Fabrication of dual stimuli-responsive multicompartmental drug carriers for tumor-selective drug release

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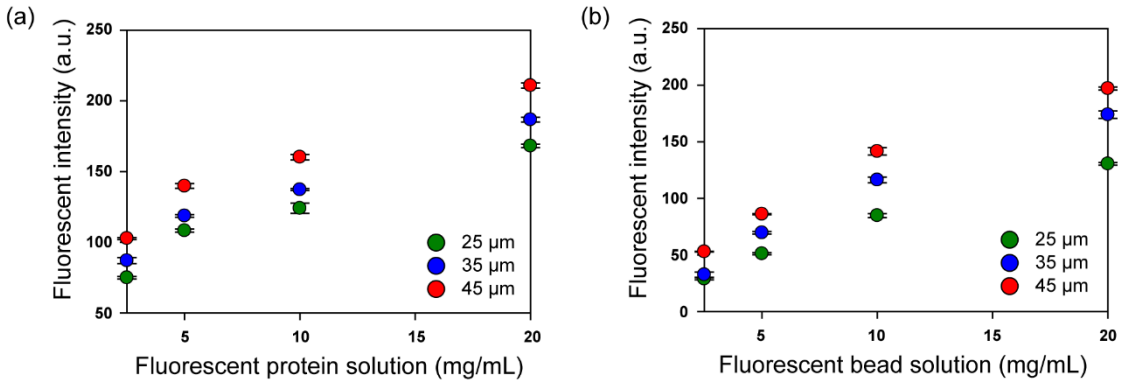

Fig. S1 Fluorescent intensities of the fluorescent model drug solutions in the microfluidic channel. (a) Rhodamine B-BSA solutions, (b) fluorescent bead solutions. Each point represents mean value from three channels. Error bars represent standard deviation.

Quantification of drug encapsulation efficiency and drug loading capacity of particles.

We quantified the drug encapsulation efficiency (EE) of the particles by comparing the fluorescent intensities of the particles with those of the model drug solutions in the microfluidic channel. The same experimental procedure was also executed in our previous study.[1] Fluorescent intensities of model drug solutions were measured in the microfluidic channels with three different heights (25 μm, 35 μm, and 45 μm). We assumed a linear relationship between the fluorescent intensity in the channel and the channel's height. As shown in Fig. S1, a linear relationship between fluorescent model drug concentration ($C_{\text{precursor}}$) and fluorescent intensity in the channel was derived for each channel's height. Using a linear equation derived from fluorescence standard curves of model drug solutions, concentrations of drugs in the particle (C_{particle}) were calculated. Then, the amount of encapsulated model drugs in the particle (M_{particle}) was calculated as follows:

$$M_{\text{particle}} = C_{\text{particle}} \times \text{volume of the particles} \tag{1}$$

To calculate EE, we considered the un-shrunken ideal volume of the particle (V_{ideal}) because rinsing steps in the microfluidic synthesis of particles lead to particle shrinkage. The area of the ideal particle was taken from the area of the photomask pattern, and the ideal height of the particle was approximately determined by subtracting the lubricant layer of PDMS (~3 μm) from the channel's height.[2] After that, EE of the particle was calculated as follows:

$$\text{EE}(\%) = \frac{M_{\text{particle}}}{C_{\text{precursor}} \times V_{ideal}} \times 100\% \tag{2}$$

EE of various particles are listed in Table S1.

We experimentally validated our calculation of encapsulation efficiency by comparing the calculated amount of model drugs in the BAC particles with the actual amount of model drugs released. To quantify the amount of model drugs released from the particles, 3500 particles were incubated with 100 mM of DTT, which leads to complete degradation of the whole particles. A percentage error between the amount of the released model drugs and the amount of the encapsulated model drugs was 14.1%. The drug loading capacity (DLC) of the particles was established as follows. To calculate wet volume of the particles, the number of prepared particles was counted by sampling 1% of the total particle solutions. Then, a hundred thousand particles were dried and weighed. If we define the density of the particles (ρ_{particle}) as the dry mass of particles over the wet volume of the particles, DLC can be calculated as the following formula:

$$\text{DLC}(\%) = \frac{M_{\text{particle}}}{\text{Mass of the particles}} \times 100\% = \frac{C_{\text{particle}}}{\rho_{\text{particle}}} \times 100\% \tag{3}$$

DLC and ρ_{particle} of various particles are listed in Table S1.

Table S1 EE and DLC of various particles fabricated with various precusor compositions and model drugs. Type 1, 2, and 3 indicate BAC particles. Type 4 indicates pH- and redox-sensitive hybrid type particles. Type 5 and 6 indicate compartments of the Janus-type multicompartmental particles.

Number of type	Height (μm)	Area (μm^2)	Fluorescent intensity (a.u.)	Model drug	$\rho_{particle}$ (g/mL)	EE(%)	DLC(%)
1	31.2	1705.4	134.6±3.3	Rhodamine B-BSA	0.171	51.1±3.6	0.60±0.04
2	41.0	2874.5	127.4±1.9	Rhodamine B-BSA	0.110	58.3±4.1	0.48±0.03
3	44.2	3054.9	125.9±3.5	Rhodamine B-BSA	0.119	49.9±8.7	0.33±0.06
4	39.5	2714.3	121.4±6.2	Rhodamine B-BSA	0.105	46.7±12.7	0.44±0.12
5	27.3	3078.2	157.9±2.4	Rhodamine B-BSA	0.171	56.7±2.0	0.87±0.03
6	40.3	5354.1	120.6±3.6	Fluorescent bead	0.125	64.1±6.3	0.53±0.05

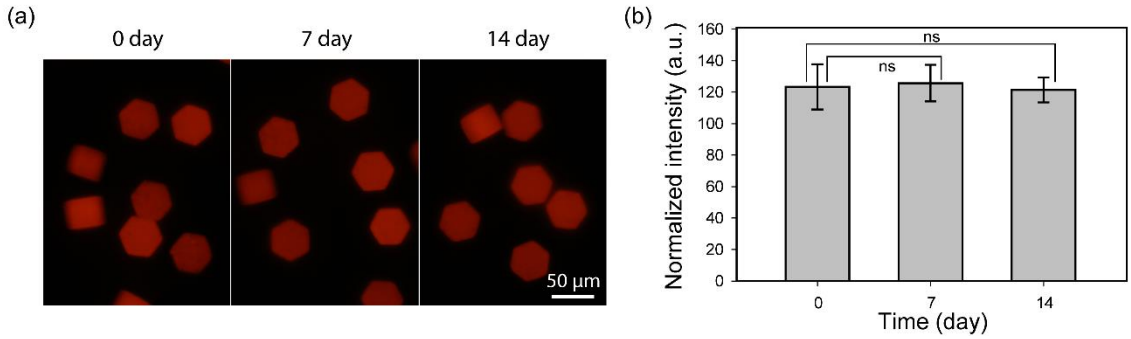

Fig. S2 Stable encapsulation of proteins in the BAC particles. (a) Fluorescent images of Rhodamine B-BSA-loaded BAC particles incuabated at pH 9.0 and 4 °C for various periods of time. The composition of the particles was identical to the one of the BAC particles as shown in Figure 2. (b) Normalized fluorescent intenstity of BAC particles upon incubation for various periods of time. Each bar represents a mean value from five particles. Error bars represent standard deviation. ns denotes $p > 0.05$.

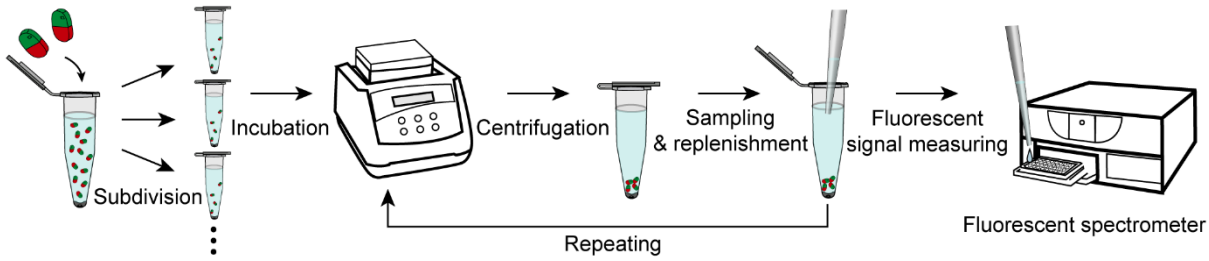

Fig. S3 Schematic diagram of the experimental procedures for the quantification of released model drugs from particles.

Cytotoxicity analysis of multicompartmental particles

Cell cytotoxicity of multicompartmental particles against HeLa human cervical cancer cells were assessed by an conventional MTT assay. HeLa cells were seeded in a 96-well plate at a density of 5×10^3 cells/well and incubated for 24 h. The cells were then incubated with multicompartmental particles at 100 μg/mL and 200 μg/mL concentrations, respectively. After 24 h or 48 h of incubation at 37 °C, the medium was replaced with fresh culture medium containing 0.1 mg of MTT powder [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazoliumbromide, Sigma-Aldrich]. After 3 h of incubation at 37 °C, formazan precipitates in the well were dissolved in DMSO. UV absorbance of the precipitate solution was measured at 560 nm to estimate the relative cell viability.

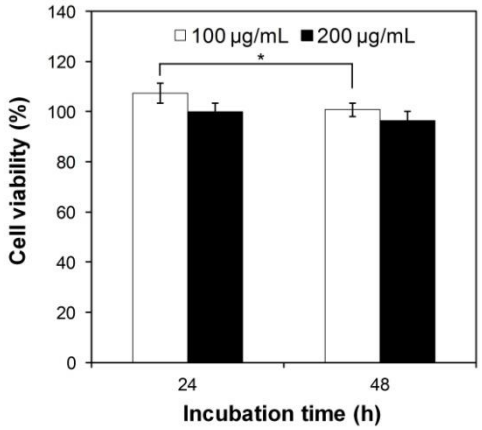

Fig. S4 Cell viability of HeLa cells after treatment with multicompartmental particles at different concentrations. Multicompartmental particles showed very low cytotoxicity against HeLa cells even at high concentrations such as 200 μg/mL. * denotes $p < 0.05$.

References

1 H. U. Kim, D. G. Choi, Y. H. Roh, M. S. Shim and K. W. Bong, *Small*, 2016, **12**, 3463-3470.

2 K. W. Bong, J. Lee & P. S Doyle. *Lab Chip*, 2014, **14**, 4680-4687.