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Mechanical stimuli responsive and highly elastic biopolymer/nanoparticles hybrid microcapsules for drug delivery applications

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Materials:
Sodium carbonate, calcium chloride, potassium chloride, magnesium chloride hexahydrate, sodium sulphate, and sodium phosphate were purchased from Sigma Aldrich and used without further purification.

(1) Synthesis of Calcium carbonate core of various sizes:
Calcium carbonate spherulites were synthesized by the modified protocol from references,[1][2] by reacting $CaCl_2$ with Na_2CO_3 using K^+ , SO_4^{2-}, Mg^{2+} ions as additives. Briefly, in a 100 mL flask, 25 ml of 200 mM Sodium carbonate was added rapidly to 25ml of 200 mM calcium chloride solution containing the additives at following concentrations: 40 mM KCl, 200 mM $MgCl_2.6H_2O$, and 200 mM of Na_2SO_4 and mixed for 5 min at 150 rpm. Then, the reaction mixture was allowed to react without stirring for 12 hrs at room temperature to get particles of ~17 μm. Smaller size particles of ~2 μm and ~10 μm were made by increasing the mixing rate 500 rpm to 1000 rpm for 5 min during the mixing of calcium chloride and sodium carbonate and reducing the reaction time to 6 hrs and 8 hrs, respectively. After reaction, particles were washed with DI water by centrifugation at 3000 rpm for three times.

[1] S. L. Tracy, C. J. P. François, H. M. Jennings, *J. Cryst. Growth* **1998**, *193*, 374.

[2] S. L. Tracy, D. A. Williams, H. M. Jennings, *J. Cryst. Growth* **1998**, *193*, 382.

Calcium carbonate core particles of various sizes

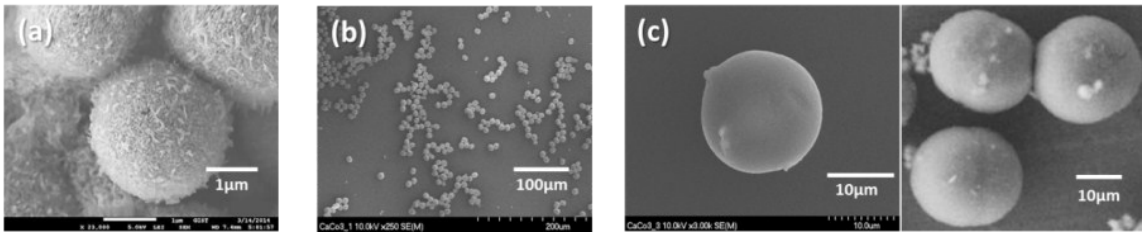

Figure S1. SEM images of various sized calcium carbonate spherulites synthesized. (a) ~2 μm, (b) ~10 μm, and (c) ~17 μm.

(2) Modification of CaCO3 with phosphate ions:

In order to render $CaCO_3$ microparticles negatively charged, 100 mg $CaCO_3$ microparticles were mixed with 10 mL of 0.2 M Na_2HPO_4 solution that was pre-adjusted to pH>12 using 0.1 M NaOH solution and mixed for 3 hrs. After reaction, the particles were washed three times with DI water by centrifugation at 3000 rpm for 3 min. The change in the surface charge from +4 mV to -13 mV proved the effective modification.

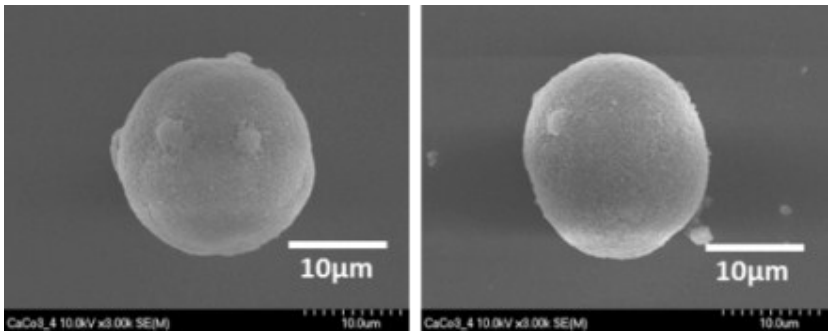

Figure S2. SEM images of phosphate modified calcium carbonate particles (CP-$CaCO_3$) of ~17 μm.

SEM images of the composites of control and HHC

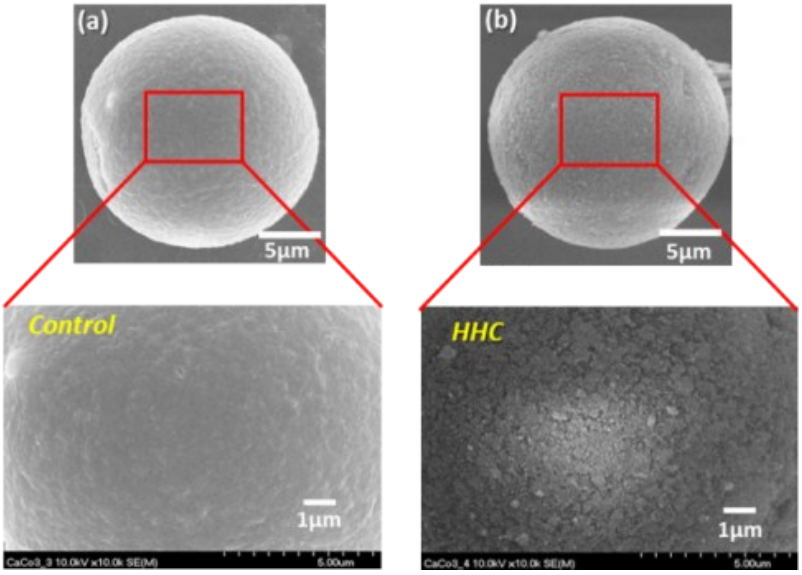

Figure S3. SEM images of (a) control composite capsule: CP-$CaCO_3$ with (Chi-Alg-Chi)-$(Alg\text{-}Chi)_3$ and (b) HHCs: CP-$CaCO_3$ with (Chi-Alg-Chi)-$(SiO_2\text{-}Chi)_3$. Scale bar: 5 µm.

<u>Zeta potential change during LbL procedure</u>

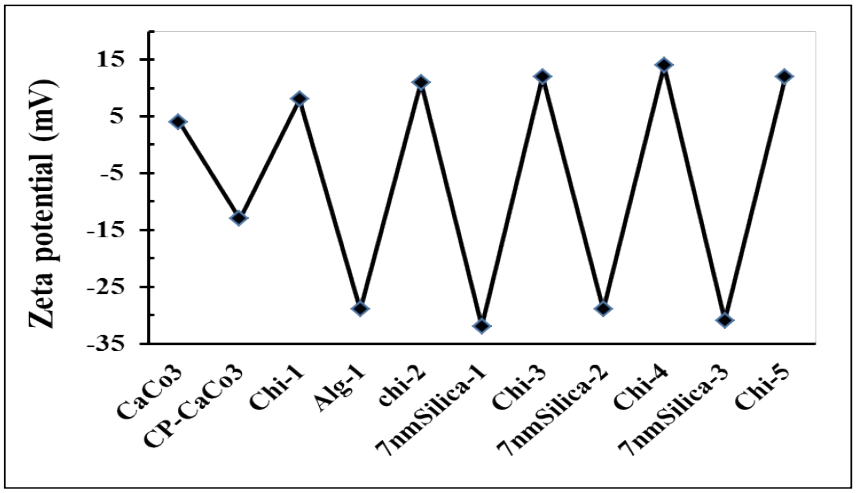

Figure S4. Zeta potential variations during each step of LbL process for preparing (Chi-Alg-Chi)-$(SiO_2\text{-}Chi)_3$ shell on $CaCO_3$ core.

SEM of HHCs and control capsules

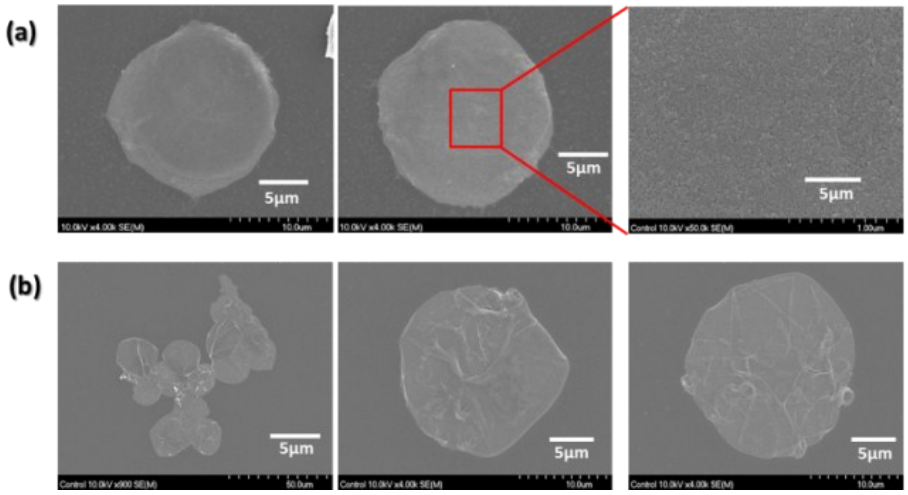

Figure S5. (a) SEM images of 3 layered (Chi-Alg-Chi)-$(SiO_2$-Chi$)_3$ HHCs and its magnified image on the right showing the presence of silica nanoparticles on the shell. (b) SEM images of control capsules (Chi-Alg-Chi)-$(Alg\text{-}Chi)_3$.

(3) Capillary micromechanics

The elastic (G) and compression modulus (k) were calculated using the following equation assuming that the static friction between the particle and capillary wall is negligible:

$$G = \frac{\frac{1}{2}(p_{wall} - p)}{\varepsilon_r - \varepsilon_z}$$

$$K = \frac{\frac{1}{3}(2p_{wall} + p)}{2\varepsilon_r + \varepsilon_z}$$

Where p-applied pressure difference; ε_r- strain deformation on radial direction; ε_z - strain deformation on axial direction;

p wall is the average pressure exerted on the capsule by the glass capillary which is given by equation

$$p_{wall} = \frac{2}{\sin(\alpha)} \frac{R_{band}}{L_{band}} p$$

Where α – taper angle of the capillary; R_{band} and L_{band} - radius and length of the band around the particle that is in contact with the glass wall respectively. Three set of experiments were done on control and 1, 3,5-L HHCs and average was taken.

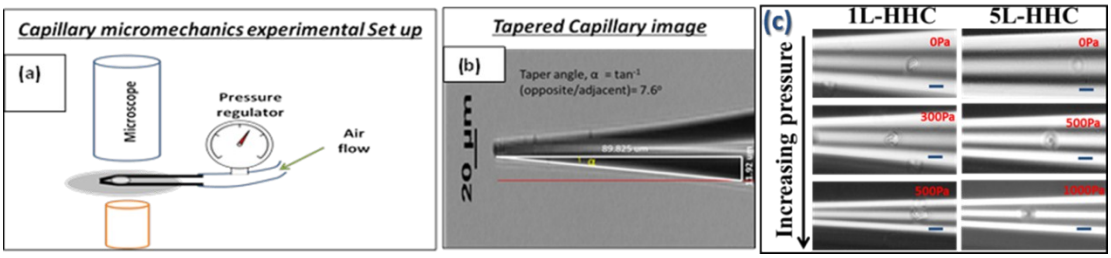

Figure S6. (a) Set-up of the capillary micro mechanics experiment; (b) Glass capillary used in the experiment tapered at 5 µm with taper angle of 7.5^{o} ; (c) Optical microscopy images of 1L-HHC and 5L-HHCs (crosslinked at -18 ^{0}C) equilibrated for 60 sec with external applied pressure mentioned on the respective images (scale bar : 20 µm).

Florescence images of osmotic pressure induced deformation of various capsules

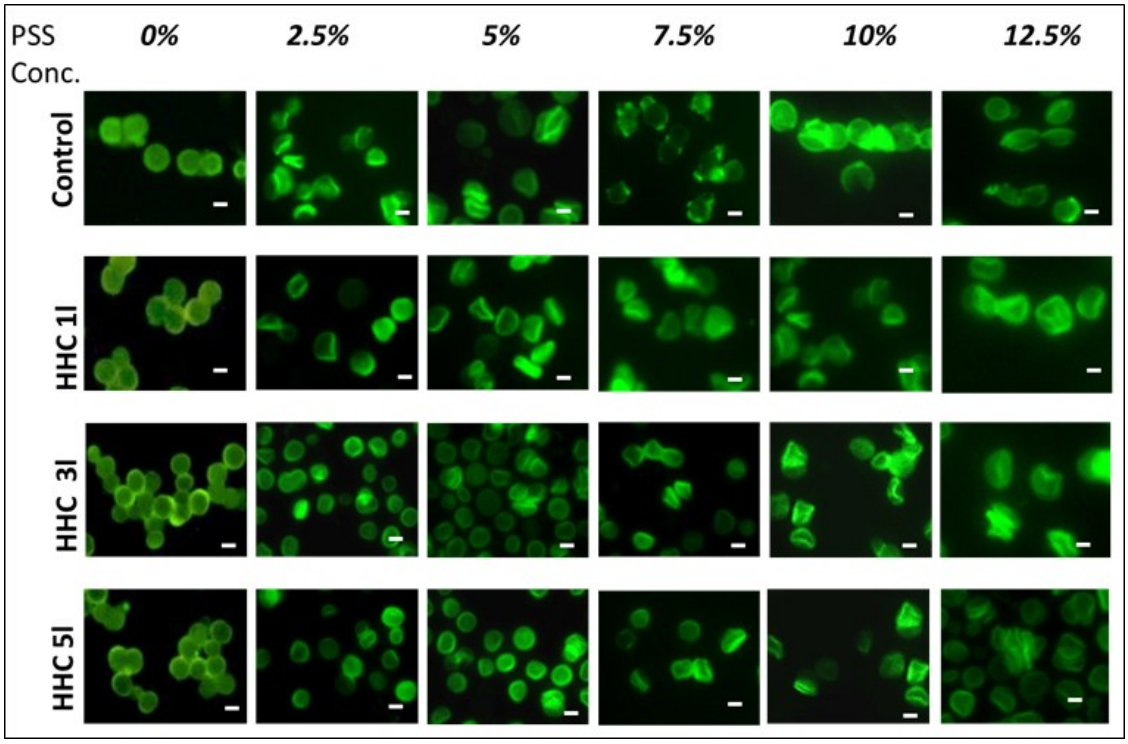

Figure S7: Fluorescence microscopy images of the control, 1L-HHC, 3L-HHs and 5L-HHCs hollow capsules at 5 different concentrations of PSS, showing the deformation behaviours of various capsules upon the external osmotic pressure induced by PSS. Scale bar: 10 µm.

SEM and AFM of various thickness capsules

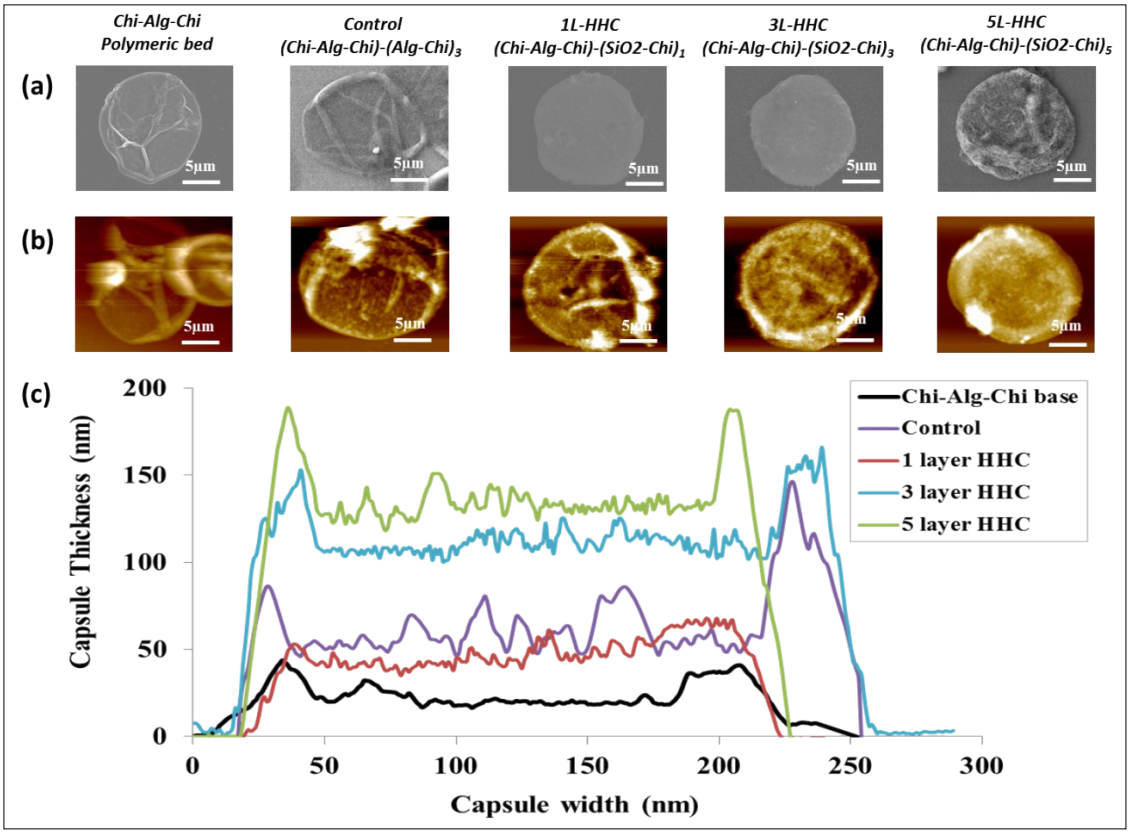

Figure S8. SEM and AFM images of the control capsule and 1, 3, and 5 SiO_2/Chitosan double layered hollow capsules crosslinked with glutaraldehyde at -18 ^{0}C for 18 hr.

Recovery of 80% deformed Control, 1L-HHC, 3L-HHC and 5L-HHC capsules

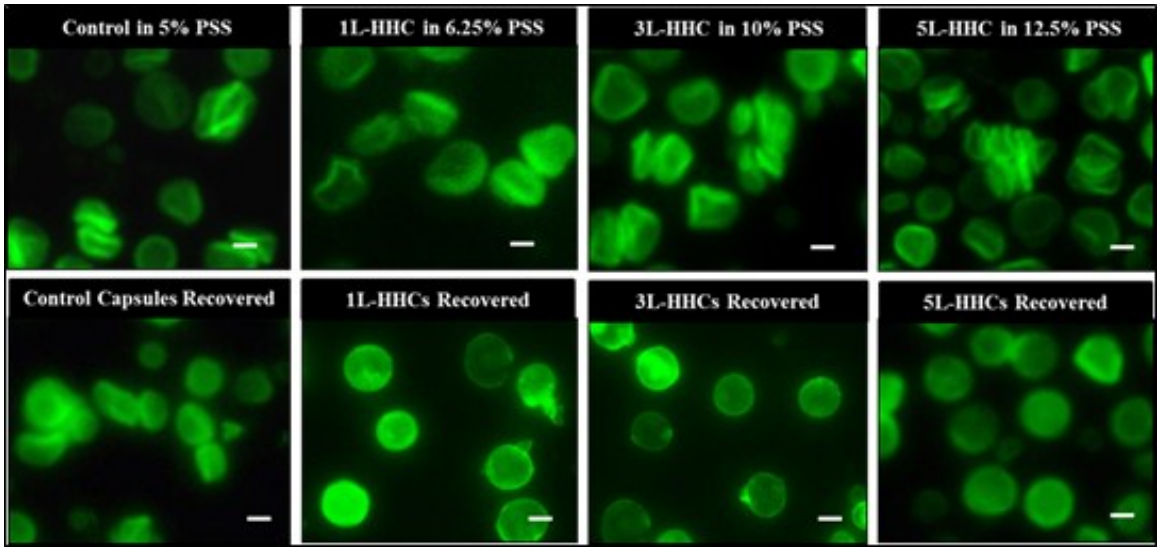

Figure S9*:* Fluorescence microscopy images of the control,1L-HHC,3L-HHC and 5L-HHC incubated with 5%, 6.25%, 10%, 11.5% and 12.5% PSS (upper panels) and the images of corresponding capsules after the removal of PSS and re-dispersion in free water after 10 min incubation (lower panels)

Stability of 3L-HHCs at different conditions

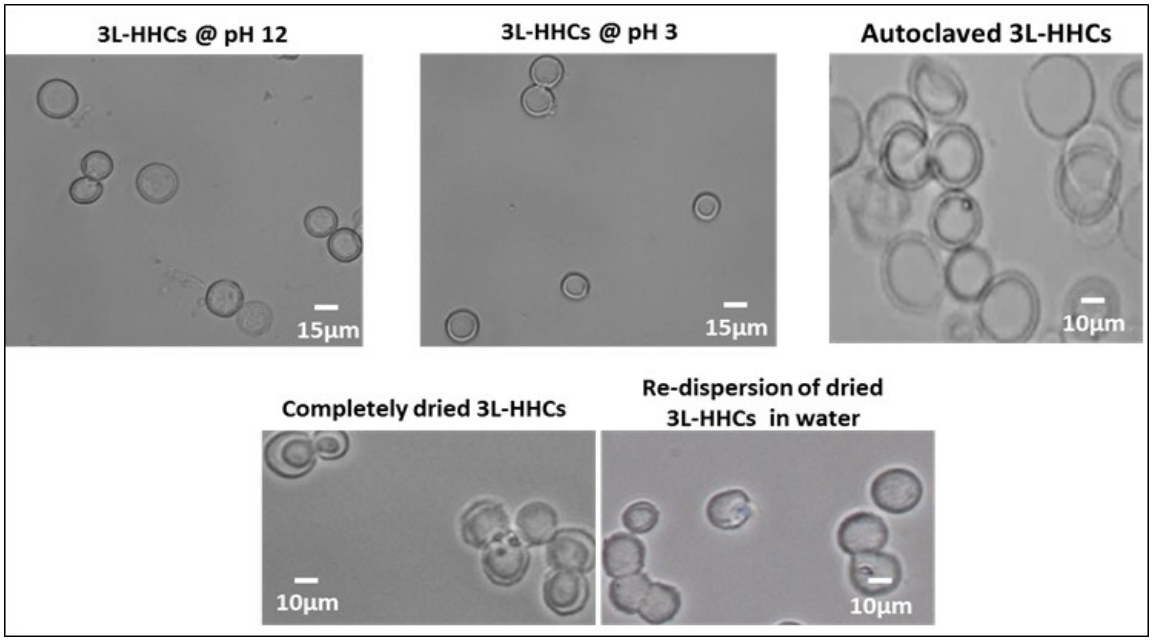

Figure S10. Optical microscopy images of 3L-HHCs (Chi-Alg-Chi)-$(SiO_2$-Chi$)_3$ showing the stability of HHCs under various conditions.

Encapsulation dyes into HHCs

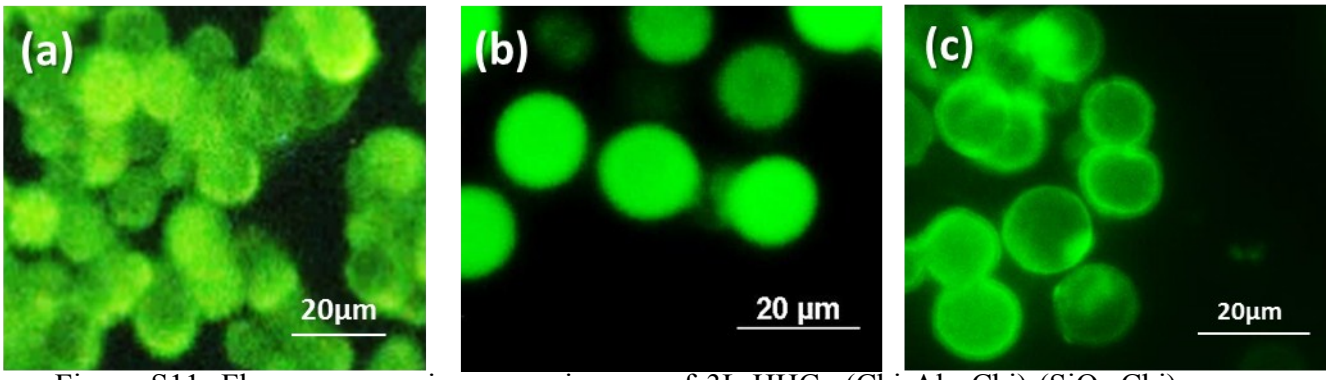

Figure S11. Fluorescence microscopy images of 3L-HHCs (Chi-Alg-Chi)-$(SiO_2$-Chi$)_3$ encapsulated with (a) FITC, (b) FITC-Dextran 4KDa, and (c) FITC-BSA.

Cytotoxicity of hybrid hollow capsules

Cytotoxicity evaluation of hybrid hollow microcapsules was carried out by the test on Extract method (according to ISO standards (ISO 10993-5, 1999) and incubating 1mg/ml 3L-HHCs onto the monolayer of NIH 3T3 fibroblast cells.

Briefly, 3T3 fibroblast cells were cultured with Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) with 10 wt % fetal bovine serum (FBS; Gibco), 100 µg/mL penicillin, and 100 µg/mL streptomycin (Gibco) as supplements in a multi-well tissue culture plates and incubated at 37 °C in 5% carbon dioxide atmosphere. The extract was prepared by incubating the sterilized capsules with DMEM culture medium without serum at 37 °C for 24 hr at an extraction ratio of 10 mg/mL. 100% extracts of the test samples as positive control and 1 mg of the hybrid hollow capsules in triplicate were placed on the monolayer of cells. After incubation of cells with extract and the capsules at 37 °C for 24 hr, cells were stained with a commercial Live/Dead assay kit, L3224 (Life Technologies, USA). After incubation at 37 °C for 30 min, the particles were washed twice by PBS and observed using fluorescence microscopy (Leica, WLD MPS32, Germany).

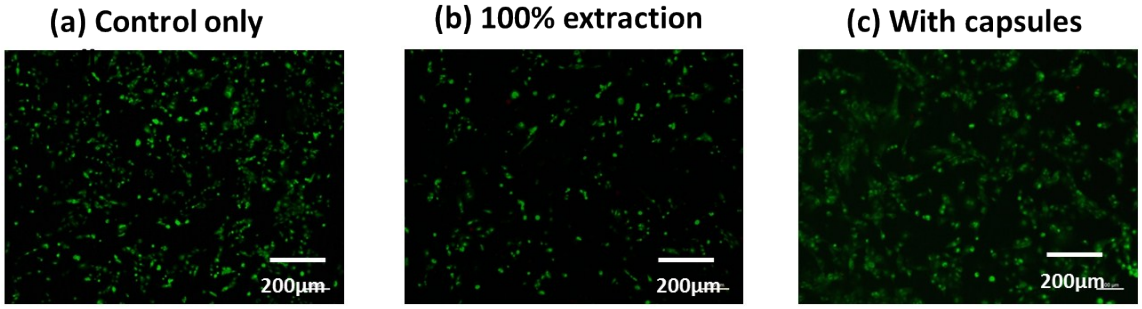

Figure S12: Cell-viability of the hybrid hollow capsules using NIH 3T3 fibroblast cells. Merged florescence Live (green)/Dead (red)) images of (a) positive control (only cells), (b) 100% extraction, and (c) incubation with 3L-HHCs (1 mg/ml) for 24 hr at 37 ^{o}C and double stained with a live/dead staining kit.

(4) Calculation of loading efficiency after model drugs into the capsules

Dried capsules ($W_{d)}$ of known weight were immersed into a dye solution of known concentration in 0.5 M NaCl at desired pH of dye dissolution and then incubated at room temperature under slight shaking for 12 hr. Then, unloaded dyes were removed by filtration (Mw cut-off of 100 kDa) and the capsules were washed with DI water once. Then, the dye loaded capsules were dispersed again in DI water and probe sonicated at 50% Amp power for 5 min to completely disperse the capsules and get rid of adsorbed dyes. The loading amounts of FITC-labelled drug (W_f) were measured by measuring the absorption at 494 nm and comparing with the standard curve. The amount of lysozyme was measured by absorbance at 278 nm, and the amount of PEI was measured by Ninhydrin assay with the absorbance at 570 nm.

The loading capacity and encapsulation efficiency of capsules were calculated from the following equations:

Loading capacity (%) = $[W_f / (W_{f+}W_d)] \times 100\%$ (Equation S1)

Encapsulation efficiency (%) = $[W_f/W_d] \times 100\%$ (Equation S2)

Table SI	Loading capacity (%)		Encapsulation efficiency (%)	
Capsule name	FITC	FITC-Dextran (4 kDa)	FITC	FITC-Dextran (4 kDa)
Control	30 ± 2	33 ± 3	43 ± 3	49 ± 6
1L-HHC	30± 1	40 ± 2	42 ± 2	65 ± 6
3L-HHC	39 ± 3	41 ± 3	64 ± 9	70 ± 7
5L-HHC	41 ± 1	40 ± 2	68 ± 4	67 ± 5

Table S1: Summary of loading capacity and encapsulation efficiency percentages of the capsules loaded with Fluorescein and FITC-Dextran 4 kDa dyes

Table S2	Loading capacity (%)				Encapsulation efficiency (%)			
Capsule name	PEI 800 Da	PEI 1300 Da	Lysozyme	FITC-BSA	PEI 800 Da	PEI 1300 Da	Lysozyme	FITC-BSA

3L-HHC	39 ± 8	36 ± 5	37 ± 6	26 ± 3	66± 8	57 ± 3	59 ± 5	36± 5
Control	32 ± 7	30 ± 3	29 ± 5	19± 9	50 ± 7	43± 6	41 ± 9	24 ± 4

Table S2: Summary of loading capacity and encapsulation efficiency percentages of the capsules loaded with various molecular weight molecules.