Sono-Assembly of Highly Biocompatible Polysaccharide Capsules for Hydrophobic Drug Delivery

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Recent advances in medicine and biotechnology have prompted the demands for developing well-defined stimulus responsive biopolymer assemblies, which can deliver precise quantities of novel therapeutics to specific target sites at desired release rate.\cite{1} Most notably, layer-by-layer (LBL) constructed polymeric multilayer capsules have demonstrated their great potential, since the assembly process allows a diverse range of materials to be incorporated into the capsule walls, a variety of cargo to be encapsulated, and the facile manipulation of capsule size, thickness, permeability, surface chemistry and surface functionality.\cite{2} The presence of multiple components in the capsule shell introduces not only multi-functionalities of capsules owing to the feasibility of combining individual polyelectrolyte properties, but also inherent drawbacks in both preparation and biomedical applications, such as tedious fabrication process, asynchronous releasing behaviour of different components at desired triggers and deteriorated biocompatibility and biodegradability in some cases.\cite{3} This continuously stimulated research to seek facile synthetic strategies for stimuli responsive capsules with single component shells.\cite{3,4}

Among those strategies, ultrasonic assembly has been of particular interest due to its “green” nature, as the assembly process relies on chemical radicals without using any chemicals like crosslinking agents,\cite{5} which are usually bio-toxic. What makes the ultrasonic assembly more attractive is that the easily biodegradable capsules assembled non-covalently can be generated in a temporarily and locally harsh ultrasonication environment.\cite{6} which has been demonstrated to be able to destroy multilayered polyelectrolyte microcapsules.\cite{7} The facile control using ultrasonication over capsules size, thickness and functionality\cite{8} encourages their biomedical applications, such as magnetic resonance imaging, echo-contrast agent for sonography and oxygen/drug delivery.\cite{10} Despite notable progress aforementioned, the development of micro- and nanocapsules utilizing ultrasonic assembly still remains a challenge, as the state-of-the-art strategy has been limited by the synthesis of mainly protein capsules.\cite{5,6,8,10} Exploring ultrasonic assembly of a wide variety of biomacromolecules and
the crosslinking mechanisms behind will advance the “green” ultrasonic synthesis of a wide variety of single component micro/nanocarriers for targeted delivery and release applications.

Motivated by the capabilities of greatly enhanced cellular uptake from polysaccharide,

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generals synthesis and potential of intracellular hydrophobic drug delivery from sono-assembled polysaccharide capsules is pursued in present work. We herein report for the first time that hydrogen bonding can be generally adopted for assembling capsules of natural polysaccharides within few minutes during sonication. Taken chitosan as the typical example in this communication, intermolecular hydrogen bonding between amino and acetoamide groups is demonstrated to stabilize the capsules, as depicted in Scheme 1. The striking shell robustness was evidenced by the long-term stability of the resulting capsules and tracing of capsule internalization by NIH3T3 cells. Importantly, the as-prepared chitosan microcapsules not only display unusual high biocompatibility, but also possess stimuli-responsiveness to local pH changes which benefits intracellular drug delivery due to negligible drug leakage at physiological pH. Moreover, synthesis of other polysaccharide molecules such as alginate can be achieved based on the same synthetic idea using hydrogen bonding during sonication. We thus anticipate that the versatility of the assembly approach will help to realize a broad variety of polysaccharides with potential for targeted therapeutic drug delivery.

Figure 1a illustrates the experimental setup for synthesis of chitosan capsules by ultrasonication. Typically, 0.5 ml vegetable oil is layered on the top of chitosan aqueous solution (pH 6.4, 4 ml), while the tip of the probe was placed at the interface between oil and aqueous solution. The sonication was carried out at 20 kHz frequency at a power density of 9 W/cm\(^2\) for 10 min in an ice bath. After sonication, a white milky suspension was obtained. Dynamic light scattering indicates that the formed microspheres have a bimodal size distribution, with two size distribution peaks situated at 1 µm and 10 µm. It should be mentioned here that the size and thickness could be tuned simply by the sonication intensities and pristine materials concentrations, as described in literatures.\[8\] Since the density of
vegetable oil is lower than that of water, microspheres with larger sizes prefer to float to the top of the solution after several days, while microspheres with smaller sizes are suspended below (Figure S1). Figure 1b shows a scanning electron microscopy (SEM) image of the prepared samples. The acquired capsules possess a smooth and crack free surface. They are deposited on the glass slide maintaining quasi-spherical shapes, indicating that the prepared containers are robust and can suffer the vacuum and coating process during preparation of SEM samples. To further demonstrate the core-shell nature of these spheres, toluene and vegetable oil were mixed prior to encapsulation, and the resulting microspheres were imaged with SEM. As the toluene is pumped away, the microspheres partially collapse but retain their structural integrity (Figure 1c). Figure 1d shows the confocal laser scanning microscopy (CLSM) image of the prepared containers loaded with Nile Red dissolved in vegetable oil, and the dye can be seen distributed evenly in the containers, indicating that hydrophobic substances can be encapsulated in chitosan microcapsules.

The presence of the chitosan shell is further proven by chemical labelling of the shell with green fluorophor (FITC), using the Schiff’s reaction of the amino group in chitosan with FITC. Figure 1e demonstrates the CLSM image of chitosan capsules modified by FITC. The green rings shown in Figure 1e clearly indicate that chitosan molecules are assembled at the surface of oil droplets and form a dense single component polysaccharide layer. The hydrophobic core and polymer shell of these spheres is important for their use in biomedical applications: the core can contain a variety of hydrophobic substances including drugs or imaging agents, and the polysaccharide shell can be easily modified to target specific organs or tumor types due to the free amino groups. It should be noted that the labelling of FITC shown in Figure 1e was done by reaction of FITC with as-formed chitosan capsules. If the modification of chitosan with FITC is done before ultrasonication, one fails to generate capsules with chitosan shell as evidenced in CLSM imaging by black spherical holes with
green background after the same ultrasonic treatment (Figure S2). This implies the importance of amino groups in the formation of chitosan capsules.

Further studies indicate that the formation of chitosan capsules is independent on the oil encapsulated inside. As seen in Figure 1f, when silicon oil is used as the core phase, spherical capsules can also be detected by SEM. Pure toluene was shown to be encapsulated by chitosan as well, and the resulting structures of capsules after natural drying on a glass slide were imaged with SEM (Figure 1g). As toluene is volatile at room temperature, the microspheres were found collapsed after natural drying, leaving ruptured shells on the glass slide. When the drying of capsules is performed at elevated temperature (60 °C) in an oven, as seen in Figure 1h, the microspheres after drying were found deformed in shape due to the air flow in the oven (Figure S3). The shell of the microspheres retained the overall structural integrity. Cavities are always found in the shell due to the fast evaporation of the toluene, as seen in Figure 1h, and the thickness of the shell could be roughly estimated to be less than tens of nanometers.

Interestingly, soft “balloon-like” shells were found for chitosan capsules by observing their shrinkage process during natural drying with optical microscopy using toluene as the oil phase. The picture sequence shown in Figure 2 discloses the shrinking process of the capsules. The whole process can be divided into two stages: isotropic shrinking stage and collapse stage. During isotropic shrinking, the behaviour of the capsule is the same as for a normal liquid droplet (Figures S4 and S5). With the loss of the toluene, the surface area of the capsule decreases. The onset of collapse takes place when the critical pressure inside the spherical shell cannot resist the external pressure.\textsuperscript{14} As seen in Figure 2, three capsules were imaged to record the collapse of their shells in the shrinking process. The buckling and collapse of the wall indicates that the stiffness of the chitosan shell is not high; however, the final remaining of the shell indicates that chitosan molecules in the shell are well cross-linked.
The result of infrared spectroscopy (Attenuated Total Reflection, ATR) shown in Figure 3a excludes the possibility of covalent cross-linking of chitosan microcapsules within the shell, since neither obvious band shift nor band appearance/disappearance were observed. However, given the lack of covalent cross-linking, the robustness of the chitosan capsule is striking as the microemulsion was found stable at room temperature over 2 months. Careful examination by ATR spectroscopy before and after sonication reveals that there is indeed no band shift except a 5 cm\(^{-1}\) one at the intense band of 1648 cm\(^{-1}\). This band relates to the C=O deformation mode of the acetoamide group,\(^{[15]}\) which derives from the incomplete deacetylation (over 75%) in industrial chitosan synthesis from chitin (Figure S6a). It has been reported that amino group and acetoamide group could be cross-linked through hydrogen bonding,\(^{[16]}\) which could evidence here the 5 cm\(^{-1}\) shift, showing the perturbation by hydrogen bonding at this band. Therefore, we propose that the intermolecular hydrogen bonding between amino and acetoamide groups is responsible for holding chitosan capsules (Figure S6b). Strong vortex mixing could not produce long-lived capsules indicating that ultrasonication plays unique roles in generating well-crosslinked chitosan capsules.

As abundant intramolecular hydrogen bonding renders chitosan low solubility in water, it is most probable that ultrasonication first breaks intramolecular hydrogen bonds, thus creating more free groups and spaces for intermolecular hydrogen bonding similar to the case in avidin capsule formation, where the S-S bonds were first destroyed and then cross-linked through ultrasonic irradiation.\(^{[9]}\) The intermolecular hydrogen bonding takes place predominantly at oil surfaces but not in bulk solutions as chitosan molecules at pH of 6.4 prefer to stay at oil/water interfaces due to hydrophobic interaction. The assumption of hydrogen bonding also explains well why modification of chitosan before sonication could not produce any chitosan shell (Figure S2): majority of amino groups are consumed by excessive FITC during modification. Owing to the limited acetoamide groups in chitosan (over 75% deacetylation), there are free amino groups remaining after shell formation, which produces functionalized green chitosan
shell in CLSM observation when chitosan capsules formed by sonication were modified with FITC. It was found that stability of capsules decreased (four weeks) when capsules were prepared through sonication of lower molecular weight chitosan (mol wt 60,000-120,000). Further experiments revealed that the proton bridging was consistent with the pH profile of capsule stability. The capsules were found stable over pHs in neutral region (from 8.5 to 5.5). When the pH value was tuned to lower than 5, the capsules were found disassembled, while capsules were found aggregated when the pH value was higher than 9. Polysaccharide capsules were found stable in low concentration salt solutions, such as sodium chloride. However, when the concentration increases up to 1 M, the capsules will become aggregated, which can be evidenced by the CLSM observations (Figure S7).

To establish the potential of chitosan capsules for hydrophobic drug delivery applications, we loaded hydrophobic coumarin 6 into the chitosan capsules by dissolving them in the vegetable oil before sonication. Fluorescence spectroscopy was used to record the release of the encapsulated coumarin 6 from chitosan capsules at different pHs by tracking the intensity evolution of maximum emission of coumarin 6 at different time periods (Figure S8). The results are shown in Figure 3b, in which obvious pH dependent release of coumarin can be observed. The releases in all cases reach different equilibrium after 200 hours, indicating the slow exchange of coumarin 6 between oil and water phases even at neutral pH. The pH dependent release is caused by pH related desorption of coumarin from capsule shell into water.[17] We calculated the release ratio at neutral pH, and found a negligible proportion (0.003‰) of coumarin 6 from chitosan capsules (Figure S9). The normalization of the three release curves (Figure S10) indicates that the release rate of coumarin 6 at high pH is lower than that at low pH. This is possibly because at pH around the isoelectric point of chitosan (6.8) the compact conformation of the chitosan chains provides the chitosan shell much less porosity for diffusion of coumarin 6. As the pH moves from high to low, more amino groups of chitosan are ionized resulting in expansion of the conformation, which favours the fast
diffusion of coumarin 6. This indicates that drugs with small molecular weights can penetrate the capsules in a controlled manner. This pH dependent release is specifically important for their potential application in intracellular hydrophobic drug delivery, as the capsules would be internalized from the slightly alkaline extracellular conditions into acidic environments (pH 5–6) inside endosomal/lysosomal compartments,\textsuperscript{[18]} which would on the other hand minimize the therapeutic side effects in blood circulation. The pH related release implies as well the potential of the carriers in therapy of tumor/cancer due to their specific feature of low pHs.

We further investigated the suitability of assembling other polysaccharides by ultrasonication. It was found that alginate capsules can be obtained using the same sonication procedure. CLSM (Figure 3c) and SEM (Figure 3d) observations reveal the formation of core-shell structured alginate capsules. The alginate microemulsion was found stable over 1 month at room temperature as well. Based on the initial experimental results, the hydrogen bonding between carboxyl groups in alginate was assumed responsible for holding the capsules, which will be reported in details in future. The results of chitosan and alginate systems greatly imply that ultrasonic assembly could be adopted as a general method for synthesizing polysaccharide capsules through a network of hydrogen bonding interactions once the polymer chains are grafted with functional groups such as carboxyl, amino, and acetoamide.

The biocompatibility of the obtained capsules in living systems is an important issue for their biomedical applications. Different amounts of capsules were added into the wells containing NIH3T3 cells. After 24 h and 48 h of incubation, the cell viability was determined by the MTT assay. Tetrazolium dye MTT is reduced to purple formazan due to the activity of cellular enzymes which can be determined with spectroscopy at 570nm.\textsuperscript{[19]} As shown in Figure 3e, the cells can survive in the chitosan container solution even at a concentration as high as 1.5 mg/ml. It is surprising to find that the cell viabilities after incubation in different amounts of capsules are always higher than 125%, indicating an excellent biocompatibility of chitosan microcapsules. 100% cell viability corresponds to a MTT result for untreated cells.
Alginate microcapsules were also tested using the same procedure for their biocompatibility. The MTT assay also reveals high biocompatibility of the alginate capsules, and the cell viability after incubation in different amounts of alginate capsules is always higher than 105% (Figure 3f). It becomes reasonable for the high biocompatibility when one considers that both the shell (polysaccharide) and core (vegetable oil) of the capsules can be nutrition for cell growth and proliferation. As cell membranes are negatively charged, chitosan capsules (positively charged) have higher chances to be intracellularly taken up than alginate capsules (negatively charged). The high biocompatibility of the microcapsules would make them good candidates for biomedical applications.

We further trace the internalization of the chitosan capsules labelled with Nile Red to clarify the enhancement of cell viability. The capsules were incubated with NIH3T3 cells in culture media for 4, 28, and 52 hours at 37 °C (5% CO₂) respectively. Before each CLSM observation, the medium containing chitosan capsules was removed, the cells were rinsed twice and a fresh culture medium was added. Subsequently, the cells were imaged directly in a glass-bottom petri dish by using CLSM. The CLSM scan normal to the dish plane (in the z-direction) revealed that the uptake of the colloidal spheres with red colour is clearly visible as point-like spots of luminescence in the cells (Figure 4a). These patterns of signals are almost in agreement with an endocytic or phagocytic mode of internalization. With increasing incubation time, more chitosan capsules were internalized as evidenced by the increased red emission, and the chitosan capsules undergo degradation, as it can be observed that the red colour becomes more spread inside the cells. One can also observe the proliferation of the cells after the incubation for 28 h and 52 h (Figure 4b and 4c), which confirms the increased cell viability shown in Figure 3e. To further determine the location of microcapsules in the cells, we used a red FM 4–64 marker to stain cell membranes and endosomes formed around the chitosan capsules during endocytosis. Yellow point-like spots of luminescence inside cells were observed due to the overlapping of the green fluorescence of the chitosan capsules.
and the red-stained endosomes (Figures 4d-4e). This provides direct evidence for endocytosis of chitosan capsules. The internalization shown in Figure 4 further proves the robustness of the chitosan shell even if it is constructed by non-covalent hydrogen bonding, and this result clearly indicates the potential application of chitosan capsules in intracellular drug delivery.

In summary, we have demonstrated general synthesis and intracellular hydrophobic drug delivery of sono-assembled single component polysaccharide capsules. We find that hydrogen bonding can be utilized as a general synthetic idea to achieve the assembly of polysaccharide in the presence of ultrasonication. Striking robustness of the capsules is evidenced by the long-term preservation of the microemulsion at room temperature and the tracing of intracellular uptake by NIH3T3 cells. Intermolecular hydrogen bonding between amino and acetoamide groups is proposed for stabilizing the capsules. In vitro investigation reveals pH dependent release of the capsules, and a negligible hydrophobic drug release was found at physiological pH. The high biocompatibilities of the capsules has been found based on the greatly enhanced cell viability in MTT assay test and evidenced cell proliferation from endocytosis observations. The free amino groups together with high biocompatibility and negligible drug leakage at neutral pH renders chitosan capsules good candidates for drug delivery with the potential of targeting specific organs or tumours. We believe our study represents a significant finding, as this is the first study of intracellular hydrophobic drug delivery of sono-assembled single component polysaccharides capsules. The versatility of the present method for assembling a wide range of polysaccharide capsules is highly anticipated to advance future rational design of capsules of diverse architectures with tailored composition for a wide range of diagnostic and therapeutic applications.
Experimental Section

Materials: Chitosan (mol wt ~600000), phosphate buffer solution (PBS), vegetable oil, coumarin 6, FITC, Dulbecco’s Modified Eagles Medium (DMEM), Glucose, Gentamicin, Thiazolyl Blue Tetrazolium Bromide (MTT), Sodium dodecyl sulfate (SDS), DMSO, acetic acid and molecular probe of FM 4-64 were purchased from Sigma-Aldrich (Germany). Calf Sera was purchased from PAA (Germany). The water used in all experiments was prepared in a three stage Millipore Milli-Q plus 185 purification system and had a resistance higher than 18.2 MΩ•cm.

Preparation and Characterization of Polysaccharide Capsules: Four milliliters of chitosan solution (pH 6.4, 5% wt) was put into a cylindrical vessel and overlaid with 0.5 ml of vegetable oil. The vessel was then attached to a sonicating probe (GEX 600, Sonics & Materials, Newtown, CT, USA). The tip of the sonicator probe was placed at the oil–water interface, and the assembly was maintained in an ice cooling bath. Typically, the sonication was carried out at 20 kHz frequency at a power intensity of 9 W/cm\(^2\) for 10 min in an ice bath. After sonication, the obtained white milky suspension was put in a dialysis tube (12-14 kDa), which was then transferred into 2L aqueous media with pH value of 6.4. The dialysis was done overnight and repeated three times to remove un-crosslinked chitosan. The size of the microspheres was measured by dynamic light scattering (Malvern Instruments, England). The morphology of samples was measured by a Gemini Leo 1550 scanning electron microscope (SEM). Confocal fluorescence images were taken by a confocal laser scanning system from Leica (Wetzlar, Germany) equipped with a 100 X oil immersion objective with a numerical aperture of 1.4. The photoluminescence spectra were recorded in a Fluoromax-4 Spectrofluorometer (150 W Xenon lamp) with the excitation wavelength of 420 nm to quantify the released drug. ATR measurement was used to determine any changing of chemical groups in chitosan before and after sonication.
In vitro Biocompatibility Study: The biocompatibility of the polysaccharide capsules was evaluated by measuring the viability of NIH3T3 cells in the presence of different concentrations of the chitosan capsules. The cells were seeded in 24 well plates at a density of 1.1 x 10^5 cells per well and incubated with capsules at various concentrations in 1 ml growth media (DMEM containing 4.5 g/L glucose, 10 v% Calf Sera and 10 µg/mL Gentamicin). In case of control sample, no capsules were added in the well. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO\textsubscript{2} in an incubator (Binder). Viability of the cells was determined by an MTT assay after 24 and 48 hours of cell culture. A stock solution of MTT (5 mg/ ml) in PBS was prepared and sterile filtered. Cells were cultured with 1mL fresh medium containing 100 µL MTT stock solution in incubator for 4 hours. Afterwards the supernatant was removed, and 1 mL of a formazan dissolving solution (99.4mL DMSO + 10g SDS + 0.6mL Glacial Acetic Acid) was added. The solutions on the cells were incubated for 5min without motion and for 5 min by slowly shaking at room temperature. After formazan was dissolved the solutions were transferred to 96 well plates and the absorbance at test wavelength of 570 and reference wavelength of 630 nm was immediately read on a microplate reader (Multiscan Ascent from Thermo Fisher). Biocompatibility of the prepared nanocontainers was expressed as percentage of cell viability, which was calculated from the ratio between optical density at (570nm – 630nm) of cells treated with the capsules and that of untreated cells (control). Data are shown as the mean ± the standard error from three independent experiments performed in triplicate.

Intracellular uptake of the capsules: For cell-growth in vitro experiments NIH3T3 cells were seeded in concentration of 6 x 10^4 cells/cm² in a 35 mm glass-bottom petri dish in DMEM cell culture medium supplemented with 10 v% Calf Sera and 10 µg/mL gentamicin. Cells were maintained as described above in an incubator (Binder) for 24 h. Then, 150 µl suspension of chitosan capsules was added to the NIH3T3 cells with 2 mL growth medium. Cells with
capsules were cultured further and characterized directly by a CLSM at different time periods of 4, 28, and 52 hours. To determine the location of chitosan capsules in the cells, we used a red FM 4–64 marker to stain cell membranes and endosomes according to the manufacturer's protocol. Subsequently, the cells were incubated in 150 μl suspension of chitosan capsules in 2 ml growth medium for 48 h (37 °C + 5% CO2), followed by three washes with PBS and direct observation via CLSM.

_In vitro drug release test:_ The release of drugs from chitosan capsules was performed in aqueous solution with different pHs. 1 ml of chitosan capsules loaded with drugs was added to dialysis tubing (12-14 KDa) and immersed into drug-free buffer solution (100 ml). Fluorescence spectroscopy of the solution was used to record the release of the encapsulated coumarin 6 from chitosan capsules at different pHs by tracking the intensity evolution of maximum emission of coumarin 6 at different time periods. The release experiments were conducted at room temperature. During the release, 3 ml of medium sample was taken out at a desired period and subjected to fluorescence measurement. After collecting the fluorescence spectra, the measured sample was put back into the stirring solution for further release test.

**Supporting Information**
Supporting Information is available online from the Wiley Online Library or from the author.

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Figure captions:

Scheme 1. Schematic illustration of intermolecular hydrogen bonding between amino and acetoamide groups that is responsible for holding chitosan capsules.

Figure 1. (a) Schematic illustration of the experimental setup for polysaccharide capsules fabrication; FESEM images of the chitosan capsules using (b) vegetable oil and (c) 4:1 toluene/oil as the oil phases with evaporated toluene part; (d) CLSM image of chitosan capsules loaded with Nile Red dissolved in vegetable oil; (e) CLSM image of chitosan capsules modified by FITC. (f) chitosan capsules fabricated using the silicon oil as the core part. FESEM observation after (g) natural drying and (h) drying in oven (60 °C) of chitosan capsules loaded with toluene as the oil phase.

Figure 2. Optical microscopy image sequence shows the shrinkage process and the buckling of the chitosan capsules using toluene as the oil phase. Scale bar: 10 μm.

Figure 3. (a) ATR spectra of chitosan capsules before and after ultrasonication using toluene as the oil phase; (b) Release profiles of chitosan capsules under different pHs; (c) SEM image of alginate capsules using vegetable oil as the core part; (d) CLSM image of alginate capsules loaded with coumarin 6; biocompatibility of (e) chitosan capsules and (f) alginate capsules measured by MTT assay.

Figure 4. Distribution of chitosan capsules loaded with Nile Red dyes in 3T3 cells, (a) 4 h, (b) 28 h, and (c) 52 h. CLSM images of magnified 3T3 cells after incubation of chitosan capsules in the presence of FM 4-64, (d) endocytotic capsules labeled with coumarin 6 (e) stained endosomes and (f) overlay image.
Figure 1
Figure 2
Figure 3
Figure 4
Cells like sugar. Motivated by this, general synthesis and potential of intracellular hydrophobic drug delivery of single component polysaccharide capsules are pursued. The capsules can be generally assembled through hydrogen bonding networks but show striking shell robustness. The evidenced cell internalization, stimuli-responsiveness to local pH changes and high biocompatibilities of the capsules specifically favor for their potential intracellular drug delivery.

Keyword: Sono-capsules

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Highly Biocompatible Polysaccharide Capsules for Hydrophobic Drug Delivery
Supporting Information

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Figure S1 Optical image of the as-obtained microemulsion after sonication of chitosan-vegetable oil. After several days’ preservation microspheres with larger sizes float on the top of the solution, while microspheres with smaller sizes suspended below.
Figure S2 CLSM image of microemulsion after sonication of FITC-labelled chitosan and vegetable oil.
Figure S3. SEM image of chitosan capsules using toluene as the oil phase after drying in an oven (60°C).
Figure S4 Optical microscopic image sequence showed the isotropic shrinkage of pure toluene droplets in water. Scale bar: 20 µm.
Figure S5 Optical microscopic image sequence showed the isotropic shrinkage of chitosan capsule using toluene as the oil phase (noted by the white arrow) in water. Scale bar: 10 µm.
Figure S6 (a) Molecular structure of chitin containing abundant acetoamide groups; (b) Intermolecular crosslinking of chitosan (75% deacetylation) through hydrogen bonding.
Figure S7 CLSM images of (a) chitosan capsules; (b) chitosan capsules in the presence of 0.1 M sodium chloride solution; (c) chitosan capsules in the presence of 1 M sodium chloride solution.
Figure S8 (a) Photoluminescence of coumarin 6 in water with various relative concentrations.

(b) Linear relationship between maximum emission intensity of coumarin 6 and the relative concentration.
Figure S9 Fluorescence spectra of chitosan capsules loaded with coumarin 6.
Figure S10 Normalized release curves of chitosan capsules loaded with coumarin 6 under different pHs.