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Switching shape of hollow layer-by-layer hydrogel microcontainers†

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Abstract

We present a novel type of ultrathin hydrogel microcapsules with pH-triggered shape switch. The capsules are produced as hollow hydrogel replicas of cubical inorganic templates and capable of keeping cubical geometries at neutral pH but transform into bulged structures at basic pH.

Shape is an important property for biological objects and plays a crucial role in biological functions. In drug delivery particle shapes affect cellular uptakes and vascular dynamics.¹ Worm-shaped polymer particles showed negligible phagocytosis compared to spheres of the same volume.² Non-spherical particles can align or tumble in the presence of flow, thus reducing their clearance in the liver or spleen.^{3,4} Oblate particles adhere more efficiently than spherical particles to the vascular endothelium of the same volume, the feature crucial for targeted delivery.⁴ A microphage internalizes an elliptical particle from the pointed end in a few minutes, while it did not succeed for several hours when attached to a flat region of the elliptical disk.⁵

The dynamic control over the bio-object properties is a key tool in the complex biological milieu. Cells switch their geometry in response to physico-chemical stimuli to achieve advanced properties and functions.⁶ Erythrocytes possess the extraordinary ability to undergo reversible large deformation and fluidity.⁷ To mimic the cells, liquid crystalline polymers have been used to prepare shape switching nanoparticles.⁸ Recently, PLGA particles switching from an elliptical disk to a sphere upon changes in temperature or pH have been reported.⁹ However, biological compatibility of the reported systems still remains a crucial issue.

Softer, flexible particles are expected to minimize phagocytosis and have a longer lifetime in the body. For instance, the prolonged circulation of worm-like micelles in the blood was in part attributed to their soft and flexible nature.² In this regard, polymeric capsules made *via* layer-by-layer (LbL) sequential assembly of polymers through electrostatic, hydrogen-bonded and covalent interactions have a strong potential as a facile platform for controlled drug delivery and related application in biotechnology.¹⁰ The LbL capsules consist of an ultrathin multilayer shell (<50 nm), which can be further functionalized, and a cavity of a sub- or micron size for loading of functional compounds. Unlike other delivery systems, capsules have high loading capacity and can deliver cargo on demand when made of stimuli-responsive polymers.^{11,12}

†Electronic supplementary information (ESI) available: Experimental details on synthesis of manganese carbonate cubic microparticles, fabrication of hydrogel PMAA capsules, CLSM, SEM, ellipsometry and XPS data. See DOI: 10.1039/c1cc12960k

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Overall, the current studies have focused on optimizing capsule chemistry and sizes with relatively little attention devoted to capsule shape. The vast majority of the capsules known to date are spherical.¹⁰ There are only few works on non-spherical structures. Rhombohedral capsules have been recently introduced *via* the LbL approach and showed to maintain the original template after core dissolution.¹³ Computer simulations predict that anisotropic capsules should exhibit greater stability under flow compared to spherical capsules.¹⁴ Hollow LbL polymeric replicas of anisotropic biological structures such as erythrocytes or *E. coli* bacteria have been also obtained.¹⁵

However, all the studies on non-spherical capsules known to date have been done with systems which are not sensitive to environmental stimuli thus focusing on maintaining shapes under various conditions. In one study pH-sensitive hydrogen-bonded LbL capsules were produced, but they disintegrated at pH>6.^{13a} Thus, to the best of our knowledge, microcapsules with dynamic stimuli-triggered switch in shape have not been reported.

Herein we demonstrate the first example of flexible hollow microcontainers with pH-switchable geometries. By using pH-sensitive ultrathin hydrogel capsules of poly(methacrylic acid) (PMAA), we investigate pH-triggered change of capsule geometry from cubical to bulged structures. The capsules are fabricated as hollow hydrogel replicas of cubical inorganic templates. For that, cubical manganese carbonate templates were coated with LbL hydrogen-bonded films of PMAA/poly(*N*-vinylpyrrolidone) (PMAA/PVPON) followed by PMAA chemical crosslinking and core dissolution as described below.

Manganese carbonate particles have been selected because of a better control over their size and cubical shape during synthesis unlike CdCO₃ and CaCO₃ microparticles, and because of their solubility in acidic solutions. The MnCO₃ particles were synthesized by a two-step procedure by mixing a MnCO₃ nano-seed solution with MnSO₄ solution followed by adding bicarbonate ammonia at 80 °C. The critical parameters to obtain monodisperse cubical particles are the ratio of components for the nano-seed solution and MnSO₄ solution volume. We found that particles size is reduced by decreasing the MnSO₄ component in the nano-seed solution while keeping the same solution volume (see details in ESI†). The SEM analysis revealed non-porous smooth surfaces of the cubical MnCO₃ particles of 5 ± 0.5 μm in size (Fig. 1a and Fig. S1b in ESI†).

Poly(ethylene imine) (PEI) was deposited first to facilitate the hydrogen-bonded multilayer growth on the cubic cores as the manganese particles possess almost no surface charge (−0.56 mV) under the deposition conditions. The ability of PEI to induce surface charge and provide enhanced adhesion of the following hydrogen-bonded multilayer was reported earlier and is due to its strong non-electrostatic contributions to the adsorption energy.^{13a} The following hydrogen-bonded (PMAA/PVPON)_{*n*} multilayer was constructed *via* LbL assembly at pH = 3.6 (0.01 M) through a well-established centrifugation method.¹⁶ For fluorescent visualization, PMAA tagged with Alexa Fluor 488 (PMAA–Alexa) was synthesized and deposited in the last bilayer (see ESI† for labeling details). To convert the hydrogen-bonded coating into a layered hydrogel, (PMAA/PVPON)_{*n*}-coated MnCO₃ particles were treated with ethylenediamine for covalent crosslinks between PMAA layers. Previously, it has been demonstrated that spherical hydrogel (PMAA)_{*n*} capsules can be obtained by this method when initially templated on spherical silica cores.¹⁷ The stability of the coating around the cubical manganese particles after crosslinking is confirmed by confocal microscopy (CLSM) (Fig. 1b and Fig. S2 in ESI†).

†Electronic supplementary information (ESI) available: Experimental details on synthesis of manganese carbonate cubic microparticles, fabrication of hydrogel PMAA capsules, CLSM, SEM, ellipsometry and XPS data. See DOI: 10.1039/c1cc12960k

Hollow hydrogel (PMAA)₁₄ capsules were then obtained by dissolving cubical MnCO₃ cores in 1M HCl solution followed by rinsing with pH=5.0 (0.01 M). CLSM in transmitted light mode evidences the complete dissolution of the cores (Fig. S3 in ESI†).

Remarkably, we have found that the (PMAA)₁₄ hydrogel capsules maintain the cubical geometry after core dissolution (Fig. S3c in ESI†). The single wall thickness of the produced (PMAA)₁₄ capsules is 22 ± 2 nm (1.6 nm per PMAA layer) as found with ellipsometry for dry (PMAA)₁₄ films fabricated under the same conditions. The results are in good agreement with an ~3.8 nm bilayer of PMAA/PVPON coating previously reported on CdCO₃ crystals of ~10 μ m and with ~40% PVPON content in the initial film.¹⁷ Considering the ultrathin capsule wall and the highly hydrated state of the PMAA network (50% water by volume as defined with liquid ellipsometry), the capsules represent a unique example of flexible but mechanically robust anisotropic hydrogel structures capable of preserving shapes of initial templates.

Next, the pH-dependent behaviour of the cubical (PMAA)₁₄ capsules was studied using CLSM by exchanging buffer in a Lab-Tek chamber (see details in ESI†). We found that the cubical geometry is retained upon exposure of the capsules from neutral to basic pH (pH = 8). Both, capsule size and cubic shape were unchanged. The capsules were then treated with EDTA solution (0.1 M). In a drastic difference to non-treated capsules, EDTA-treated capsules transform from cubical into the bulged structures at pH = 8. Fig. 2 contrasts pH-triggered behaviour of hydrogel capsules before and after EDTA treatment at pH = 8. The CLSM image of non-treated capsules shows flat facets with well-defined corners and edges (Fig. 2a). In contrast, the capsules wrinkle along the initially cubical plane after EDTA treatment (Fig. 2b). The 3D CLSM images evidence that untreated capsules retain the cubical shape at pH = 8 but became convex with geometry close to spherical after EDTA treatment (Fig. 2c and d).

It was previously reported that spherical hydrogel (PMAA) capsules, initially templated on spherical silica cores, responded to pH variations by undergoing a 2-fold increase in size when exposed from neutral to basic pH.¹⁷ The latter was explained by pH-triggered swelling of capsule walls. (PMAA)_n hydrogel films shrank at neutral pH but swelled at basic pH due to repulsion between ionized carboxylic groups.¹⁷

In the case of hydrogel cubical capsules, we observe that the capsules exhibit pH-triggered change in shape but only after EDTA treatment. To study the mechanism of the shape switch, the pH-responsive properties of PMAA hydrogels were tested before and after EDTA treatment.

First, we investigated the surface charge of the capsules before and after EDTA treatment by measuring zeta-potential of the capsule suspension under various pH values (see details in ESI†). Fig. 3 shows that the zeta-potentials of the untreated (PMAA)₇ capsules are similar at pH=5 and pH=8 with the values of -5.2 ± 4.4 mV and -7.9 ± 5.3 mV, respectively. In contrast, the zeta-potential of the EDTA-treated capsules at pH = 8 is highly negative, -22 ± 8.7 mV. Apparently, positively-charged manganese ions trapped in the capsule walls after core dissolution decrease the negative charge at pH 8 by binding with ionized carboxylic groups. However, the EDTA treatment results in chelation and subsequent removal of the ions out of the capsule walls. As a result, negative charge increases due to the increased number of available –COO– groups. The XPS analysis of the capsules before and after EDTA treatment reveals the 8-fold decrease in the manganese ion concentration within the walls after EDTA treatment confirming the zeta-potential data (Fig. S5 in ESI†).

We found that change in shape from cubical to bulged structures was not accompanied by any detectable changes in sizes as tested with confocal microscopy. In control experiments

we fabricated spherical (PMAA)₁₄ capsules by dissolving spherical MnCO₃ cores. Size analysis illustrates that the spherical capsules retain similar sizes at pH = 5 and pH = 8 before EDTA treatment. In contrast, EDTA-treated spherical capsules exhibited a 2-fold increase in size when pH is increased from pH = 5 to pH = 8 (Fig. S3a in ESI[†]). In the case of cubical capsules, it was technically more challenging to follow pH-triggered size variations due to complex geometry and high flexibility of the capsule walls.

Finally, we investigated the effect of manganese ions on swelling behaviour of surface-attached LbL (PMAA)₁₄ films upon pH variations by using liquid ellipsometry (see details in ESI[†]). The films do not reveal any pH-induced changes in thickness in the presence of manganese ions in the films. In contrast, the EDTA-treated hydrogel films clearly demonstrate a 10-fold increase in thickness when pH is changed from pH = 5 to pH = 8 (Fig. S4b in ESI[†]). The result is in good agreement with the increased diameters of the spherical capsules observed under these conditions (Fig. S3a in ESI[†]).

Overall, our results suggest that manganese ions suppress pH-responsive behaviour of hydrogel systems which is recovered after removal of the ions with EDTA. Indeed, manganese ions restrain pH-induced swelling and change in size for hydrogel films and spherical capsules, respectively. As a result of recovered pH-induced wall swelling, EDTA-treated cubical capsules exhibit shape change due to bulging at pH = 8. One might expect that the capsules switch back to their initial shape at pH=5. Indeed, pH-dependent swelling of the hydrogel film and dimensions of the spherical capsules are reversible (Fig. S4 in ESI[†]). However, once “bulged”, the cubical capsules remain as such. Apparently, the swollen wall is too soft to recover the initial capsule shape. Indeed, the (PMAA) hydrogels showed a significant decrease in stiffness upon swelling at high pH.¹⁸ The studies on the effect of hydrogel stiffness on the shape reversibility are currently under investigation.

In summary, we demonstrated a novel type of ultrathin anisotropic hydrogel capsules produced as LbL polyacid replicas of cubic inorganic microparticles. The capsules preserve the geometry of the initially cubical template under both acidic and basic conditions after core dissolution. However, the capsules transform into bulged structures at basic pH after being treated with EDTA. The geometry of the capsule is controlled by the manganese ions trapped in the capsule wall after core dissolution. The capsule geometry transformation occurs due to the removal of the ions with EDTA. The hydrogel capsules of pH-tunable geometry hold a significant promise as novel smart carriers for controlled drug delivery. The shape-adjustable macro-containers also open new opportunities in pH-regulating transport behaviour in microfluidic devices, a feature we are currently exploring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
A SEM image of MnCO_3 cubical particles (a). A CLSM image in a fluorescence mode of $(\text{PMAA})_{14}$ -coated MnCO_3 particles (b).

Fig. 2. CLSM (a, b) and 3D reconstruction images (c, d) of (PMAA)₁₄ hydrogel capsules at pH=8 before EDTA (a, c) and after EDTA (b, d) treatment.

Fig. 3. pH-dependent variations of zeta-potential of (PMAA)₇ capsules before (open columns) and after EDTA treatment (filled columns).