Synthesis of Nonspherical Microcapsules through Controlled Polyelectrolyte Coating of Hydrogel Templates

Lynna Chen,† Harry Z. An,‡ and Patrick S. Doyle*,‡

†Department of Biological Engineering and ‡Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

Supporting Information

ABSTRACT: We report a simple approach to fabricate custom-shape microcapsules using hydrogel templates synthesized by stop flow lithography. Cargo-containing microcapsules were made by coating hydrogel particles with a single layer of poly-l-lysine followed by a one-step core degradation and capsule cross-linking procedure. We determined appropriate coating conditions by investigating the effect of pH, ionic strength, and prepolymer composition on the diffusion of polyelectrolytes into the oppositely charged hydrogel template. We also characterized the degradation of the templating core by tracking the diffusivity of nanoparticles embedded within the hydrogel. Unlike any other technique, this approach allows for easy fabrication of microcapsules with internal features (e.g., toroids) and selective surface modification of Janus particles using any polyelectrolyte. These soft, flexible capsules may be useful for therapeutic applications as well as fundamental studies of membrane mechanics.

INTRODUCTION

Microparticles and microcapsules with customizable shape, mechanical flexibility, and surface anisotropy have received attention in recent years for their role in biomedical applications ranging from drug delivery to cell mimics for fundamental studies in flow.1–5 Various techniques for the controlled synthesis of nonspherical microparticles have been developed,6,7 including flow lithography,8 nonwetting template molding (PRINT),9 electrohydrodynamic jetting,10 droplet microfluidics,11 and film stretching.12 However, solid microparticles are often inefficient at carrying cargo for therapeutic or diagnostic purposes. An ideal carrier would be a cargo-filled capsule with optimized shape and flexibility, as exemplified by the body’s natural delivery vehicle—the red blood cell. However, synthetic microparticles with these properties remain a challenge to fabricate due to limitations in template shape or material chemistry.

Several groups have fabricated nonspherical microcapsules by coating sacrificial templates using layer-by-layer techniques (LbL) and subsequently removing the core. Templates include solid polymer microparticles,13–15 sacrificial biological entities such as red blood cells,16,17 and microparticles made from inorganic materials—both porous (e.g., calcium carbonate,18 manganese carbonate,19 silica20) and nonporous (e.g., silicon21). Shortcomings of these templating strategies include restricted geometries and difficult loading of active therapeutics due to material incompatibility or processing conditions. Alternatively, spheroidal capsules have been made through arrested coalescence or mechanical squeezing of particle-decorated droplets.21–25 For this approach, the range of shapes generated is very limited, resulting suspensions are often polydisperse, and capsule sizes are usually very large (i.e., 100–1000 μm).

Stop flow lithography (SFL), a technique that combines microfluidics with photolithography, can be used to fabricate hydrogel microparticles with any 2D-extruded shape in a high-throughput manner.26,27 Previous work has shown that this technique can be used to create anisotropic striped particles with different chemical compositions27 and entrap biologics and small molecule therapeutics in the biocompatible and easily functionalizable hydrogel matrix.28,29 These properties make SFL microparticles attractive candidates for microcapsule templates.

In this work, we create custom-shape microcapsules using SFL particle templates, which can encapsulate nanoscale cargo. We coat microparticles (10–200 μm in size) using a single polyelectrolyte layer, creating core–shell structures with micron thick shells created by diffusion of the polyelectrolyte into the oppositely charged hydrogel matrix. We demonstrate how we can subsequently dissolve away particle cores, leaving behind mechanically flexible hollow capsules that retain complex template geometries. We show how to control the diffusion of the polyelectrolyte into the particle matrix, and hence the effective shell thickness, by tuning parameters including pH, ionic strength, and hydrogel prepolymer composition.

Our approach enables easy surface modification of nonspherical hydrogel particles that can be applied to a variety of polyelectrolytes, with the capability to selectively functionalize...
Janus or patchy particles. With one additional step, we can fabricate hollow microcapsules in any lithographically defined shape. In contrast to the majority of capsules formed by LBL, our capsules only require deposition of a single polyelectrolyte layer, eliminating many time-consuming coating and rinsing steps. To our knowledge, this is the first demonstration of microcapsules fabricated using hydrogel templates with customizable shape and size.

**Experimental Section**

**Materials.** Poly(ethylene glycol) diacrylate (M<sub>n</sub> = 700 g/mol), acrylic acid (anhydrous, ≥99.0%), poly(ethylene glycol) (M<sub>n</sub> = 200 g/mol), 2-hydroxy-2-methyl-1-phenylpropan-1-one (Darocur 1173), poly-L-lysine hydrobromide (M<sub>n</sub> = 150–300 kDa), hydrochloric acid (37%), N-(3-(dimethylaminopropyl))-N'-ethyldiaminediethanol chloride (≥99.0%), dimethyl sulfoxide (≥99.9%), and glycerol (≥99%) were purchased from Sigma-Aldrich and used as received. Fluorescein isothiocyanate dextran sulfate sodium (M<sub>n</sub> = 46 000 g/mol) was purchased from TdB Consultancy. Fluoresbrite carboxy YG microspheres (2.6% solids in water, diameter = 0.19 μm, λ<sub>em</sub> = 441/486 nm) were purchased from Polysciences. Methacryloyl-thiocarbonyl rhodamine B (λ<sub>em</sub>/λ<sub>ex</sub> = 548/570 nm, Polysciences) and cyanine 5 N-hydroxy succinimide (λ<sub>em</sub>/λ<sub>ex</sub> = 646/662 nm, Lumiprobe) were used for fluorescent labeling. PBST was made with 1X phosphate buffered saline (without calcium and magnesium, Corning) and 0.05% (v/v) Tween 20 (Sigma-Aldrich). Sodium chloride (crystal, Mallinckrodt Pharmaceuticals), sodium bicarbonate (powder, J.T. Baker), and sodium hydroxide (pellet, Macron Fine Chemicals) were dissolved in deionized water before use. Poly-L-lysine (PLL) was covalently attached to microbeads using EDC coupling (30:20 μL of a well-mixed solution containing 10% particles in PBST and 90% (v/v) 1 M NaOH was loaded into chambers made from a glass slide and a coverslip separated by two parallel strips of parafilm. The chamber was completely sealed using UV-cured optical glue (865, Norland) to prevent drift and evaporation. Videos of 300–500 frames were taken using an EB-CCD camera (CCD9304, Hamamatsu) connected to an inverted microscope (Axiovert 200, Zeiss) at a rate of 29.6 frames/s at room temperature. A public-domain MATLAB algorithm written by Kilfoil and co-workers was used to identify fluorescent beads and track their two-dimensional trajectories (Figure S4). Trajectories were averaged to obtain the mean-squared displacement (MSD) as a function of lag time. Static error due to camera noise was determined by analyzing immobilized beads. Particle tracking accuracy was verified by analyzing videos of freely diffusing 200 nm polystyrene beads in water. The bulk diffusivity calculated from the MSD of beads in water matched the theoretical value (within 5% error).

**RESULTS AND DISCUSSION**

**Polyelectrolyte Deposition on Hydrogel Templates.** Figure 1 illustrates our general approach to create core–shell structures, selectively coat Janus particles, and synthesize microparticles in the desired photomask-defined shapes (Figure 1A) using a prepolymer solution containing PEGDA, acrylic acid (AAc), and photoinitiator. The carboxyl groups from the AAc (pK<sub>a</sub> ~ 4.5<sup>55</sup>) are covalently linked to the cross-linked PEG hydrogel, creating a negatively charged matrix at neutral pH. After collecting and washing the particles, we coated them with the biocompatible polycation, poly-L-lysine (PLL, M<sub>n</sub> = 150–300 kDa), by immersing the particles in an aqueous solution of PLL with controlled pH and ionic strength. At neutral pH, the cationic PLL (pK<sub>a</sub> ~ 10.5<sup>55</sup>) interacts with the...
anionic hydrogel particle via electrostatic interactions (Figure 1B). As a result, the PLL forms a uniform shell on the particle by diffusing into the outer edge of the hydrogel matrix (Figure 1C). We can then deposit additional layers of oppositely charged polyelectrolytes (e.g., dextran sulfate (DXS)) or dissolve away the template core to form hollow microcapsules. We show a sampling of particle morphologies achievable using this technique in Figure 1D−G. We demonstrate uniform PLL coating of a particle of topological genus 2 (shape with two holes) (Figure 1D), two-layer (PLL/DXS) coating of a three-dimensionally patterned shape fabricated by grayscale lithography (Figure 1E), and selective PLL coating of a Janus particle (Figure 1F). In Figure 1G, the template core has been removed to form a microcapsule encapsulating 200 nm fluorescent nanoparticles as model cargo.

This technique can be used for region-specific coating of Janus particles fabricated using two streams of prepolymer—one with acrylic acid and one without. As previously described,70 Janus or multistripe particles can be easily fabricated by SFL using conflowing laminar streams in a multi-inlet microfluidic device. The PLL selectively coats the negatively charged portions of the particles, allowing for spatially controlled functionalization of hydrogel microparticles (Figure 2).

In addition to partially coated Janus particles, we can form fully coated core−shell structures with shell thicknesses around 3−4 μm using a PLL solution with neutral pH and ionic strength between 0.5 and 1 M NaCl. We define shell thickness by the full width at half-maximum (fwhm) extracted from fluorescence intensity profiles of confocal images of Cy5-PLL coated particles. Shells are formed by diffusion of the PLL into the hydrogel matrix, which differs from conventional nanometer scale LBL layers that are built on top of nonporous substrates.35,36 The extent of diffusion of PLL into the matrix depends on a combination of steric and electrostatic effects.37

We observed the contribution of steric effects by altering PLL molecular weight and PEGDA concentration. At pH 7/0.5 M NaCl, PLL$_{150}$−300 kDa diffuses into the entire hydrogel, while PLL$_{150}$−300 kDa (used in the majority of this work) forms a shell (Figure S1), similar to what is observed by Bysell and co-workers in a PLL/poly(acrylic acid) microgel system.38 Also as expected, increasing PEGDA concentration decreases the mesh size of the gel39 and consequently decreases the thickness of the shell by limiting the diffusion of PLL into the hydrogel (Figure 3D, at constant pH of 7 and ionic strength of 0.5 M NaCl).

As shown in Figure 3A,B, increasing the ionic strength from 0 to 1 M NaCl (pH held constant at 7) decreases the extent of PLL diffusion into the particle, creating thinner and more well-defined shells. These thin shells are believed to result from two physical processes. First, there is a reduction in the hydrogel mesh size due to decreased swelling of the PEGDA-AAc particle (Figure S2) by the Donnan effect.37,41 Second, the charge screening at higher ionic strength enables denser packing of PLL molecules in the outer region of the particle, which sterically prevents further inward diffusion of other PLL molecules. A similar trend was observed by Kaufman et al. during the fabrication of polyelectrolyte microcapsules across water/oil droplet interfaces.42 As expected, polyelectrolyte deposition at extremely high ionic strength (i.e., 5 M NaCl) is unsuccessful due to complete charge screening.

At very low or very high pH (<2 or >12), the hydrogel or PLL is respectively no longer charged, and no PLL is deposited
in either case. At a pH close to the pK₅ of the hydrogel matrix (ionic strength held constant at 0.5 M NaCl), the PLL completely diffuses into the particle, while at a pH range where both the PLL and the PEGDA-AAc are charged, a thin shell forms (Figure 3C). This suggests that inward diffusion is impeded by an increase in charge density of one of the interacting species. High charge density of PLL or PEGDA-AAc results in immediate complexation of the two species that prevents further inward diffusion of PLL, while the longer reaction time scale for less densely charged species allows PLL to penetrate further into the hydrogel matrix. Changing the pH of the PLL deposition solution (C; [NaCl] = 0.5 M, PEGDA = 50%), the pH of the PLL solution (C; [NaCl] = 0.5 M, PEGDA = 50%), the pH of the PLL deposition solution (B; pH = 7, PEGDA = 50%), and the PEGDA concentration (v/v) of the prepolymer solution (D; pH = 7, [NaCl] = 0.5 M), N = 10 (error bars represent standard deviation). Scale bars are 30 μm.

Figure 3. Controlling the diffusion of PLL into hydrogel templates. Confocal micrographs and fluorescence intensity profiles across the indicated cross sections show the diffusion of Cy5-PLL (MW: 150–300 kDa) into PEGDA-AAc hydrogel particles for different ionic strengths (A). The extent of diffusion of PLL into the particle is determined by steric effects and the strength of the electrostatic interaction between PEGDA-AAc and PLL. Shell thicknesses, defined as the fwhm extracted from fluorescence intensity profiles, are plotted as a function of the ionic strength of the PLL deposition solution (B; pH = 7, PEGDA = 50%), the pH of the PLL solution (C; [NaCl] = 0.5 M, PEGDA = 50%), and the PEGDA concentration (v/v) of the prepolymer solution (D). Micrographs and fluorescence intensity profiles are plotted as a function of the ionic strength of the PLL deposition solution (B; pH = 7, PEGDA = 50%), the pH of the PLL solution (C; [NaCl] = 0.5 M, PEGDA = 50%), and the PEGDA concentration (v/v) of the prepolymer solution (D; pH = 7, [NaCl] = 0.5 M), N = 10 (error bars represent standard deviation). Scale bars are 30 μm.

Figure 4. Degradation of hydrogel templates to form PLL capsules. Uncoated and Cy5-PLL (MW: 150–300 kDa) coated hydrogel particles are immersed in 0.9 M NaOH and monitored with fluorescence microscopy over time (rhodamine acrylate dye is copolymerized into the hydrogel) (A, scale bars are 30 μm). Degradation of the PEGDA-AAc core is due to base-catalyzed hydrolysis of ester bonds in the hydrogel (B). Low-magnification fluorescent image shows intact PLL capsules after 64 h incubation in NaOH (C, scale bar is 200 μm).

Degradation of Hydrogel Templates to Form Microcapsules. To make hollow microcapsules, we simply immerse hydrogel particles with a single-layer PLL shell in basic conditions (0.9 M NaOH) to degrade the particle core via ester hydrolysis (Figure 4). While uncoated particles completely disintegrate under these conditions, PLL shells remain intact (Figure 4A). The particles swell during the hydrolysis process due to osmotic pressure caused by the breakup of the cross-linked polymer into smaller fragments (Figure 4B). After 26 h, the capsules begin to revert back toward their initial size and shape when fully cleaved degradation products are able to diffuse through the remaining membrane, causing osmotic pressure to decrease. Quantification of capsule dimensions during the degradation process shows that capsules eventually return to their photomask-defined sizes, demonstrating the precise control over capsule shape and size achievable using this technique (Table S1). The capsules remain intact for more than 2 weeks in hydrolytic conditions (pH 14). To quantify the degradation process, we performed particle tracking on hydrogel microparticles synthesized with embedded 200 nm diameter carboxylate polystyrene beads. Particle tracking is commonly used to interrogate the evolving mechanical properties of cells using beads embedded in the cell cytoplasm. More recently, particle tracking has been used to monitor hydrogel degradation via metalloproteinases. In our study, the beads were physically trapped in the polymerized PEGDA-AAc hydrogel particle, initially static due to the tight
mesh of the intact hydrogel. As the hydrogel is degraded in NaOH, the gel porosity increases and the beads begin to move (Figure 5A). The plot of the beads’ mean-square displacement (MSD) at long time points also show that only beads in the center of the microcapsules diffuse and that beads embedded within the PLL shell remain static. The diffusing beads remain confined within intact microcapsules for more than 2 weeks (see Video S1).

The fluorescent polystyrene nanoparticles represent model cargo that can be encapsulated within SFL-templated microcapsules. It should also be possible to encapsulate smaller cargo, down to tens of nanometers in size, by tuning the pore size of the capsule membrane. This may be achievable through denser packing of PLL controlled by deposition conditions, by adjusting the molecular weight of both the PEGDA and the PLL, or by adding additional coating layers. Changing the permeability of the capsule membrane may add additional functionality for encapsulation and controlled release of different compounds. Capsules have been observed to release their contents when exterior stress causes the membrane to rupture. Possible reasons for membrane rupture include adhesion to glass slides or other capsules, osmotic shock due to changing ionic strength of the surrounding solution, or mechanical forces that cause extreme capsule deformation. Our results suggest that microcapsules can be used as containers to encapsulate various types of cargo that can be embedded within the hydrogel template and that this cargo can remain trapped until the capsule disintegrates or ruptures. Although our current technique uses harsh conditions for template core removal, we can alter our hydrogel chemistry to allow for mild degradation conditions suitable for encapsulation of biologically relevant cargo. For example, we can fabricate hydrogel templates using a diacrylated poly(lactic acid)–PEG block copolymer that has been shown to degrade in physiological conditions after 1 week, as demonstrated in previous work from our group.49

Since microcapsules retain the complex shapes of their lithographically defined templates, we are able to make novel capsule shapes that cannot be easily fabricated with any other technique. For example, Figure 6 shows genus 1 and 2 microcapsules, similar to shapes observed in phospholipid bilayers as demonstrated in previous work from our group.49

![Particle tracking videos](image)

**Figure 5.** Quantifying hydrogel degradation over time using particle tracking. 200 nm diameter YG carboxylate polystyrene beads embedded into hydrogel particles are monitored by particle tracking after immersing Cy5-PLL-coated particles in 0.9 M NaOH. Mean-squared displacements (MSDs) of beads in the center of hydrogel particles (inset) are plotted as a function of lag time (τ) for various time points in the core degradation process. MSDs in bulk for freely diffusing beads in water are plotted for comparison (A). MSDs at τ = 0.1 s are plotted with respect to time immersed in NaOH (the red curve is a fit to the data, described by the given equation) (B). The dashed line on both plots represents the MSD for immobilized beads due to static noise.38 Scale bar for inset is 20 μm.

![Microcapsules](image)

**Figure 6.** Microcapsules made from templates with internal features. Genus 1 and 2 particles are coated with Cy5-PLL, and the template cores are subsequently dissolved in 0.9 M NaOH to form capsules. Particles are imaged by confocal fluorescence microscopy before (A) and during core dissolution after 19 h in 0.9 M NaOH (B). Swelling in NaOH due to osmotic pressure causes stretching of the outer membrane and buckling of the inner membrane. A close-up shows detail of the buckled inner membrane (C). Cross-sectional views (YZ and XZ) are shown for the slices indicated by the white lines in the XY view. Scale bars are 50 μm.
vesicles.\textsuperscript{50} The genus 2 capsule is a double-holed torus. Confocal images of swollen microcapsules show that osmotic pressure in these genus 1+ capsules leads to expansion of the outer membrane and buckling of the inner membrane (Figure 6B,C). This enables future studies of mechanical properties of thin polymer membranes.\textsuperscript{51,52}

The deformation of capsule membranes in response to osmotic stress is one demonstration of the flexible nature of these hollow microcapsules. To further test their deformability, we collected microcapsules after complete core removal (9 days in 0.9 M NaOH) and loaded them into a microfluidic channel with a contraction (channel height: 60 \( \mu \)m, wide section: 300 \( \mu \)m wide \( \times \) 3300 \( \mu \)m long, contraction: 30 \( \mu \)m wide \( \times \) 300 \( \mu \)m long). Figure 7 shows a representative example of a rectangular microcapsule (63 \( \mu \)m wide \( \times \) 89 \( \mu \)m long, 58 \( \mu \)m in height) deforming to pass through the contraction under manually applied pressure and recovering its original shape on the other side of the contraction. We believe that these flexible capsules have potential to make good model systems for studying nonspherical capsule flow dynamics.\textsuperscript{53}

After observing the stability and mechanical flexibility of these microcapsules, we sought to determine the mechanism for how these capsules remain intact under harsh basic conditions. Since the hydrogel templates are coated by PLL via electrostatic interactions, we anticipated that the majority of nonspherical capsule under immersion in NaOH for particles without EDC coupling but remains the same for PLL-coated particles with EDC coupling. All particles are imaged by epifluorescence with the same imaging conditions and displayed with intensities normalized across all four images. The fluorescence intensity profiles for the indicated cross sections of particles in NaOH are plotted on the right (normalized to the maximum intensity of the EDC particle). Scale bars are 50 \( \mu \)m.

Figure 8 compares the fluorescence intensity for particles in PBS and 5 min after immersion in 0.9 M NaOH, under identical imaging conditions. Note that in the bottom left image of Figure 8 (no EDC, pH 14) the particle is present but undetectable when the fluorescence intensity is normalized. This provides evidence that a significant portion of PLL does immediately dissociate in highly alkaline conditions when it is not covalently linked to the PEGDA-AAc hydrogel. However, we suspect that a very small amount of PLL remains and forms amide linkages to the PEGDA-AAc, which is expected under basic conditions.\textsuperscript{57} By covalently linking to the PEGDA-AAc, the PLL acts to cross-link the shell, preventing degradation of the hydrogel matrix where PLL is present. This hypothesis is consistent with FTIR analysis of bulk samples before and after degradation for plain PEGDA-AAc, PEGDA-AAc with PLL, and PEGDA-AAc with PLL cross-linked with EDC (Figure S4). To achieve similar results, other groups have covalently linked polyelectrolyte multilayers through amide bonds using heat,\textsuperscript{58} polyelectrolytes with chemically reactive end groups,\textsuperscript{59} or EDC chemistry.\textsuperscript{60} One advantage of our current technique is the single-step simultaneous core removal and shell cross-linking procedure. Since the edges of the hydrogel template are cross-linked by the PLL to form a stable shell, a small amount of template material remains in the capsule. This is confirmed in particle tracking videos showing static beads trapped in the shell (Video S1). However, in contrast to conventional LbL capsules where complete template removal is desirable due to template incompatibility with biological systems, our PEGDA hydrogel templates are biocompatible, and remnants of the material within the shell do not limit the capsules’ use in potential applications. This remnant material could also be beneficial in some applications. For example, it can be readily functionalized with DNA and antibodies or serve to control the porosity and/or modulus of the capsule.

\section*{CONCLUSIONS}

We have demonstrated how hydrogel microparticles fabricated by SFL can be used as templates to create custom shape microcapsules via a simple and controllable polyelectrolyte deposition and core dissolution process. The unique advantages of this approach include the ability to create flexible microcapsules that retain complex (e.g., genus 1+) template geometries and the ability to selectively functionalize the surface of Janus particles. In addition, we believe that our technique can be easily modified to use mild processing conditions suitable for encapsulation of biologically active compounds. Future studies will focus on interrogating the mechanical properties of these capsules as well as investigating the controlled release of encapsulated compounds of interest. This will allow future development of these designer microcapsules for use in drug delivery, in tissue engineering, or as model systems for studying their flow dynamics or membrane mechanics.
ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.5b02200.

Figures showing (i) effect of PLL molecular weight on coating, (ii) swelling of PEGDA-AAc particles, (iii) particle tracking images, and (iv) FTIR spectra of bulk samples; table showing particle dimensions during and after template removal (PDF).

Movie showing microcapsules with encapsulated nanoparticles (AVI)

Movie showing capsule flow through a contraction (AVI)

AUTHOR INFORMATION

Corresponding Author

*E-mail pdoyle@mit.edu (P.S.D.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is supported by the Institute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred. Additional support was provided by NSF grants CMMI-1120724 and DMR-1006147. We thank Octavio Hurtado for help with device microfabrication at the BioMEMS Resource Center, Wendy Salmon for confocal microscopy training, Stephen Morton for helpful discussions, and Nima Yazdan Panah and Tim McClure for FTIR assistance. L.C. is supported in part by a postgraduate scholarship from Natural Sciences and Engineering Research Council (NSERC) of Canada.

REFERENCES

(1) Venkataraman, S.; Hedrick, J. L.; Ong, Z. Y.; Yang, C.; Ee, P. L. R.; Hammond, P. T.; Yang, Y. Y. The Effects of Polymeric Nanosphere Shape on Drug Delivery. Adv. Drug Delivery Rev. 2011, 63 (14–15), 1228–1246.

(2) Yoo, J.-W.; Irvine, D. J.; Discher, D. E.; Mitragotri, S. Bio-Inspired, Bioengineered and Biomimetic Drug Delivery Carriers. Nat. Rev. Drug Discovery 2011, 10 (7), 521–535.

(3) Mitragotri, S.; Lahann, J. Physical Approaches to Biomaterial Design. Nat. Mater. 2009, 8 (1), 15–23.

(4) Shimoni, O.; Yan, Y.; Wang, Y.; Caruso, F. Shape-Dependent Cellular Processing of Polyelectrolyte Capsules. ACS Nano 2013, 7 (1), 522–530.

(5) Best, J. P.; Yan, Y.; Caruso, F. The Role of Particle Geometry and Mechanics in the Biological Domain. Adv. Healthcare Mater. 2012, 1 (1), 35–47.

(6) Glotzer, S. C.; Solomon, M. J. Anisotropy of Building Blocks and Their Assembly into Complex Structures. Nat. Mater. 2007, 6 (8), 557–562.

(7) Dudukuri, D.; Doyle, P. S. The Synthesis and Assembly of Polymeric Microparticles Using Microfluidics. Adv. Mater. 2009, 21 (41), 4071–4086.

(8) Dudukuri, D.; Pregibon, D. C.; Collins, J.; Hatton, T. A.; Doyle, P. S. Continuous-Flow Lithography for High-Throughput Micro-particle Synthesis. Nat. Mater. 2006, 5 (5), 365–369.

(9) Rolland, J.; Maynor, B.; Euliss, L.; Exner, A.; Denison, G.; DeSimone, J. Direct Fabrication and Harvesting of Monodisperse, Shape-Specific Nanobiomaterials. J. Am. Chem. Soc. 2005, 127, 10096–10100.

(10) Roh, K.; Martin, D. C.; Lahann, J. Biphasic Janus Particles with Nanoscale Anisotropy. Nat. Mater. 2005, 4 (10), 759–763.

(11) Shum, H. C.; Abele, A. R.; Lee, D.; Studart, A. R.; Wang, B.; Chen, C.-H.; Thiele, J.; Shah, R. K.; Krummel, A.; Weitz, D. A. Droplet Microfluidics for Fabrication of Non-Spherical Particles. Macromol. Rapid Commun. 2010, 31 (2), 108–118.

(12) Champion, J. A.; Katera, Y. K.; Mitragotri, S. Making Polymeric Micro- and Nanoparticles of Complex Shapes. Proc. Natl. Acad. Sci. U. S. A. 2007, 104 (29), 11901–11904.

(13) Morton, S. W.; Herlihy, K. P.; Shopowitz, K. E.; Deng, Z. J.; Chu, K. S.; Bowerman, C. J.; Desimone, J. M.; Hammond, P. T. Scalable Manufacture of Built-to-Order Nanomedicine: Spray-Assisted Layer-by-Layer Functionalization of PRINT Nanoparticles. Adv. Mater. 2013, 25 (34), 4707–4713.

(14) Doshi, N.; Zahr, A. S.; Blaskar, S.; Lahann, J.; Mitragotri, S. Red Blood Cell-Mimicking Synthetic Biotmaterial Particles. Proc. Natl. Acad. Sci. U. S. A. 2009, 106 (51), 21495–21499.

(15) Yan, X.; Garapty, A.; Champion, J. A. Engineering Polyelectrolyte Capsules with Independently Controlled Size and Shape. Langmuir 2015, 31 (27), 7601–7608.

(16) Shallender, M.; Luo, R.; Venkatraman, S. S.; Neu, B. Layer-by-Layer Microcapsules Templated on Erythrocyte Ghost Carriers. Int. J. Pharm. 2011, 415 (1–2), 211–217.

(17) Donath, E.; Moya, S.; Neu, B.; Sukhorukov, G. B.; Radostina, G.; Voigt, A.; Baumler, H.; Kiesewetter, H.; Mohwald, H. Hollow Polymer Shells from Biological Templates: Fabrication. Chem. - Eur. J. 2002, 8 (23), 5481–5485.

(18) Yashchenok, A.; Parakhonskiy, B.; Donatan, S.; Kohler, D.; Skirtach, A.; Möhwald, H. Polyelectrolyte Multilayer Microcapsules Templated on Spherical, Elliptical and Square Calcium Carbonate Particles. J. Mater. Chem. B 2013, 1 (9), 1223.

(19) Schepelina, O.; Lisunova, M. O.; Drachuk, I.; Tsukruk, V. V. Morphology and Properties of Microcapsules with Different Core Releases. Chem. Mater. 2012, 24 (7), 1245–1254.

(20) Kozlovskaya, V.; Alexander, J. F.; Wang, Y.; Kuncewicz, T.; Liu, X.; Godin, B.; Karfampieva, E. Internalization of Red Blood Cell-Mimicking Hydrogel Capsules with pH-Triggered Shape Responses. ACS Nano 2014, 8 (6), 5725–5737.

(21) Rozynek, Z.; Mikkelsen, V.; Dommersnes, P.; Fossom, J. O. Electroformation of Janus and Patchy Capsules. Nat. Commun. 2014, 5, 3945.

(22) Studart, A. R.; Shum, H. C.; Weitz, D. A. Arrested Coalescence of Particle-Coated Droplets into Nonspherical Supracolloidal Structures. J. Phys. Chem. B 2009, 113 (12), 3914–3919.

(23) Pawar, A. B.; Caggioni, M.; Ergun, R.; Hartel, R. W.; Spicer, P. T. Arrested Coalescence in Pickering Emulsions. Soft Matter 2011, 7 (17), 7710.

(24) Subramaniam, A. B.; Abkarian, M.; Mahadevan, L.; Stone, H. A. Colloid Science: Non-Spherical Bubbles. Nature 2005, 438 (7070), 930.

(25) Datta, S. S.; Abbaspourrad, A.; Amstad, E.; Fan, J.; Kim, S.-H.; Romanowsky, M.; Shum, H. C.; Sun, B.; Utada, A. S.; Windbergs, M.; et al. 25th Anniversary Article: Double Emulsion Templated Solid Microcapsules: Mechanics And Controlled Release. Adv. Mater. 2014, 26 (14), 2205–2218.

(26) Dudukuri, D.; Gu, S. S.; Pregibon, D. C.; Hatton, T. A.; Doyle, P. S. S-Stop-Flow Lithography in a Microfluidic Device. Lab Chip 2007, 7 (7), 818–828.

(27) An, H. Z.; Helgeson, M. E.; Doyle, P. S. Nanoemulsion Composite Microgels for Orthogonal Encapsulation and Release. Adv. Mater. 2012, 24 (28), 3838–3844 3895.

(28) An, H. Z.; Safai, E. R.; Burak Eral, H.; Doyle, P. S. Synthesis of Biomimetic Oxygen-Carrying Compartmentalized Microparticules Using Flow Lithography. Lab Chip 2013, 13 (24), 4765–4774.

(29) Appleyard, D. C.; Chapin, S. C.; Srinivas, R. L.; Doyle, P. S. Bar-Coded Hydrogel Microparticles for Protein Detection: Synthesis, Assay and Scanning. Nat. Protoc. 2011, 6 (11), 1761–1774.
