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Immersive Polymer Assembly on Immobilized Particles for Automated Capsule Preparation

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Polymer capsules are of significant interest for a range of biomedical applications because they can be prepared with tailored physicochemical properties, and can be engineered to encapsulate and release therapeutics and target specific cells. Layer-by-layer (LbL)-engineered particles and capsules^[1] represent a particular class of materials that have received widespread interest,^[2] as they can be prepared with different sizes,^[2-4] shapes,^[4] and mechanical strengths.^[5] These particles and capsules have also been examined for application in drug and vaccine delivery because they can be engineered to exhibit stealth,^[6] targeting,^[7] and stimuli-responsive properties.^[8] Despite the progress made in this field of research, the sequential layering process used to assemble the polymer multilayers on the precursor particles is often labor intensive and time consuming. A number of studies have focused on streamlining LbL assembly on templates by utilizing filters or microfluidic devices.^[9] However, these systems remain limited, as they do not offer the broad versatility inherent to conventional LbL assembly with regard to the choice of template particle size and exploitation of different polymer interactions. Further, issues arise from filter composition, pore size, channel width and recovery method, which lead to engineering challenges such as membrane caking, channel clogging, or a low-throughput of capsules. Recently, we

introduced the concept of immobilizing template particles in agarose gels for the rapid and versatile layering of particles using electrophoretic polymer assembly (EPA).^[10] Although EPA is useful for preparing a range of LbL-assembled capsules with useful material properties, uncharged polymers remain difficult to electrophorese, thus limiting the full range of materials and functionalities inherent to conventional LbL assembly.

Herein, we introduce an approach to prepare polymer capsules based on layering on immobilized particle templates that retains the inherent benefits of conventional LbL assembly, is applicable to uncharged polymers, allows parallel layering of different particles and/or polymers, and is easily automatable. Immobilizing sacrificial particles in a gel essentially permits the complexity of layering on three-dimensional substrates to be treated as a two-dimensional, planar scenario. Therefore, immersive polymer assembly (IPA) on immobilized particles can use standard, planar dip-layering protocols^[11] with little modification. Similar to LbL assembly on planar substrates, immersing the immobilized particles in polymer solution results in polymer assembly on the particle surface. After recovering the layered particles from agarose and subsequently removing the template particles, stable capsules of different diameters are obtained (**Scheme 1**). Furthermore, IPA can be used with a StratoSequence robotic dipper (Nanostrata Inc.) for automated LbL assembly.^[11c, d]

The biologically derived polysaccharide, agarose, was used as an immobilizing gel because the pore size is highly heterogeneous, on the order of 100 nm, and because polymers are capable of diffusing through the large agarose fibers, which are roughly 50-100 nm in diameter.^[12] However, rhodamine isothiocyanate (RITC) labeling of the agarose shows that there is also a larger pore size population with a range of 1-10 μm (Figure S1). These pores have historically been conducive to the separation of biomolecules utilizing an electric current, but they also allow for polymers and other materials to passively diffuse through the gel without significant caking.^[13] Furthermore, agarose is compatible with many buffers and

stable over a wide pH range (2-14).^[13a, c] Because LbL-capsules are of biomedical interest, it is important to note that agarose is biologically compatible, commonly used in medical research,^[14] and acts as an edible framework for many Asian desserts. Further, agarose can be melted with heat, which allows the coated template particles to be recovered by centrifugation at a yield of approximately 80%.^[10]

Differential interference contrast (DIC) microscopy and flow cytometry experiments showed that the template silica (SiO₂) particles were well dispersed both upon immobilization in agarose and after subsequent recovery from agarose (Figures S2 and S3). The first experiments were performed manually and LbL buildup via IPA was studied with electrostatically coupled polymers, namely fluorescein isothiocyanate (FITC)-conjugated poly(allylamine hydrochloride) (PAH) and sodium poly(styrene sulfonate) (PSS). Three manual washing steps between each layer proved adequate to remove excess polymer (Figure S4). After the deposition of each PAH/PSS bilayer, the coated particles were recovered by melting the agarose (~65° C) and their fluorescence intensity was assessed using flow cytometry. (Lower melting point agarose (~35° C) can also be used as the immobilizing gel.) A linear increase in fluorescence was observed for the deposition of four bilayers of PAH/PSS, which were layered in 0.5 M NaCl solution at pH 7 (**Figure 1**), suggesting uniform layer buildup. IPA was also performed on smaller template SiO₂ particles, i.e., 1.11 μm and 585 nm diameter particles. After recovering the coated particles from agarose and subsequently removing the template particles, stable capsules of different diameters were observed by fluorescence microscopy (**Figure 1(i)-(iii)**). The capsules prepared from particles layered in agarose do not retain any detectable residual agarose after recovery and core dissolution (as assessed by Fourier transform infrared spectroscopy; data not shown), which is in agreement with our previous observation on capsules prepared via EPA.^[10] Atomic force microscopy (AFM) experiments revealed that the shell thickness of the capsules was just under 5 nm, or ~1.25 nm per bilayer (Figure S5), which is approximately half of the thickness of PAH/PSS

capsules prepared using conventional LbL assembly (centrifuge/wash).^[15] This difference is likely due to the hindered diffusion of polymers in the IPA approach, leading to thinner layers.

Holes that could arise from contact points between the agarose and the templates were not visible by AFM; however the measurements were carried out in a dry state, which could make such observations difficult. To further investigate if the immobilization and recovery process left holes in the films, super-resolution optical microscopy was used to image core-shell particles in liquid. Structured illumination microscopy (SIM),^[16] with a resolution of ~125 nm for green fluorophores, did not show any apparent holes in the films (Figure S6). This suggests that if contact points exist, they would be less than ~125 nm and therefore restricted to the diameter of a single agarose fiber, which is estimated to be 50-100 nm for 1% gels.^[12b] Therefore, FITC-labeled dextrans with diameters of gyration roughly 15, 45, and 90 nm for 70 kDa, 500 kDa, and 2MDa, respectively, were used to monitor the permeability of IPA capsules using confocal laser scanning microscopy (CLSM).^[17] Interestingly, it was observed that the IPA capsules were less permeable than capsules prepared by conventional LbL assembly. Over 90% of conventional LbL capsules were permeable to 2MDa FITC-dextran, while only 25% of the IPA capsules were permeable to this size of dextran (Figure S7). Further, 50% of IPA capsules were permeable to 500 kDa FITC-dextran and nearly 75% of IPA capsules were permeable to 70 kDa FITC-dextran, while all conventional LbL capsules were permeable to FITC-dextran below 2 MDa. The impermeability of IPA capsules to molecules smaller in diameter than individual agarose fibers, combined with the improved impermeability of IPA capsules in comparison to conventional LbL capsules, possibly suggests that possible contact points between the templates during immobilization have a limited influence on the resultant capsule properties. It is also likely that the heating process during template recovery from agarose has a larger effect on the material properties of the resultant capsules, as conventional LbL capsules showed a similar decreased permeability (as

did IPA capsules) when subjected to a heating process equivalent to the recovery process of IPA-coated particles prior to core removal (Figure S8).

We next investigated different polymers for the buildup of responsive, degradable, single-component LbL capsules. Single-component LbL capsules are prepared by covalently cross-linking one polymer component in the film, and selectively releasing the sacrificial intermediate layers via a pH change. These capsules are of interest because of enhanced stability under physiological pH and their tunable degradability dictated by the choice of cross-linker. We have previously reported the preparation of functional alkyne-containing poly(2-diisopropylaminoethyl methacrylate) (PDPA_{Alk}),^[8c] and thiol-modified poly(methacrylic acid) (PMA_{SH})^[8a] capsules via conventional LbL assembly. In the current studies, layer buildup of Alexa Fluor 488 (AF488)-conjugated PDPA_{Alk} with PMA and AF488-conjugated polyvinylpyrrolidone (PVP) with PMA_{SH} was monitored via flow cytometry (**Figure 2a, b**). After recovering the particles from agarose, the PDPA_{Alk} or PMA_{SH} films were cross-linked. Stable, pH-responsive capsules were formed by template dissolution followed by removal of the intermediate layers of PMA or PVP by washing into 20 mM phosphate buffered saline (PBS) for PDPA_{Alk} and PMA_{SH}, respectively. Both the cross-linked PDPA_{Alk} and PMA_{SH} capsules displayed pH-dependent shrinking and swelling (**Figure 2a(i,ii), 2b(i,ii)**), and both were degradable under reducing conditions due to the stabilizing disulfide bridges, which is in agreement with similar capsules prepared using conventional LbL assembly.^[8a, c]

We automated the IPA process using a StratoSequence robotic dipper modified with a custom-made gel-holding basket. Multiple gels were loaded and unloaded from the basket, and the gel holding basket was attached to the robotic-dipper. The instrument was computer controlled, and run with a slightly altered dip layering protocol.^[11c, d] Four bilayer PAH/PSS capsules, and separately four layer, single-component PMA_{SH} capsules, were prepared using the robotic dipper (**Figure 3**). Three separate blocks of gel were loaded into the gel-holding

basket to test the suitability of IPA for parallel processing. Each gel contained different sized immobilized template particles, which produced template particle-sized PMA_{SH} capsules after automated IPA (**Figure 3a-c**), highlighting a distinct secondary advantage of IPA over conventional LbL assembly for the generation of capsules.

We have demonstrated that functional LbL capsules can be prepared by immersing gel-immobilized particles in polymer solution, which allows for polymer deposition and layer buildup, followed by gel and template particle removal. IPA is useful for layering polymers through different interactions (including uncharged polymers) and is capable of layering polymers on different-sized particles at the same time. Particles of different composition and shape should be equally applicable to IPA. Moreover, IPA provides capsules with reduced permeability compared with conventional LbL capsules and is useful for the hands-free preparation of responsive, degradable, single- or multiple-component capsules. The potential for scaling up, and the utilization of commercially available robotic dipping equipment, makes IPA a valuable technique for the preparation of biomedically-relevant capsules.

Experimental

Materials: Sodium poly(styrene sulfonate) (PSS, $M_w \sim 13$ kDa) was purchased from Fluka. Poly(allylamine hydrochloride) (PAH, $M_w \sim 15$ kDa), polyvinylpyrrolidone (PVP, $M_w \sim 10$ kDa), rhodamine isothiocyanate (RITC), fluorescein isothiocyanate (FITC) and FITC-dextran (70 kDa, 500 kDa, 2 MDa) were purchased from Sigma-Aldrich. Poly(methacrylic acid) (PMA, $M_w \sim 15$ kDa) was obtained from Polysciences, Inc. Alexa Fluor 488 (AF488) was purchased from Invitrogen. Poly(2-diisopropylaminoethyl methacrylate) (PDPA) containing alkyne moieties was synthesized as previously reported [8c]. Thiol-containing PMA (PMA_{SH}) was made through modification of PMA, as previously reported [8a]. Certified PCR Low Melt Agarose, was purchased from BioRad. High purity deionized water with a resistivity

greater than 18.2 M Ω .cm (Milli-Q water) was obtained from an inline Millipore RiOs/Origin water purification system. Silica (SiO₂) particles (585 nm, 1.11 μ m, 2.59 μ m, and 4.59 μ m diameter) were obtained from Microparticles GmbH. All of the buffers were made up in Milli-Q and pH adjusted with sodium hydroxide (1 M) or hydrochloric acid (1 M) accordingly. Sodium chloride at pH 7 (0.5 M), sodium acetate at pH 4 (50 mM), and phosphate buffered saline (20 mM) (PBS) were used.

Gel Preparation of Agarose Containing Silica Particles: A 1% agarose gel was prepared by weighing out agarose powder and adding a corresponding volume of buffer. 50 μ L of SiO₂ particles (50 mg mL⁻¹) of the desired diameter was added for each 1.5 mL of 1% gel solution. While liquid, this solution was cast into the desired shape and left to cool.

Sample Recovery: The agarose-containing layered particles were heated until dissolved, and the resultant solution was centrifuged at 37 °C at 800 g for 60 s to pellet the particles. The supernatant was removed and discarded and the particles were washed three more times and suspended in buffer to remove excess agarose.

Core Removal: The particles were rinsed with 13.3 M ammonium fluoride buffered with 5 M hydrofluoric acid (HF) at a volumetric ratio of 1:1.5. Capsules were then washed with Milli-Q water three times to remove all traces of HF. Caution: HF is extremely dangerous, handle with caution!

Immersive Polymer Assembly (IPA): Agarose containing SiO₂ particles was immersed in polymer solution (0.5 mg mL⁻¹) for 15 min. This was followed by a wash step consisting of immersion in Milli-Q water for PAH or PSS layers, or buffer for PDPA, PVP or PMA_{SH} layers, for 5 min, three times. The process was repeated for each polymer layer until the desired number of layers was achieved.

Automated IPA: A StratoSequence robotic dipper was programmed using the commercial StratoSmart software to dip in polymer solutions for 15 min, dip in washing solutions for 5 min, and rotate at low speed while immersed in all solutions. The layer number was set to 8,

and the machine was run. The whole process took just over 4 h and ran without any interference. A custom gel holder was made by weaving wire mesh from a colander into the shape of a cup. The bottom of this custom gel holder was sparsely threaded wire that permitted the free flow of water and did not retain excess water once removed from solution.

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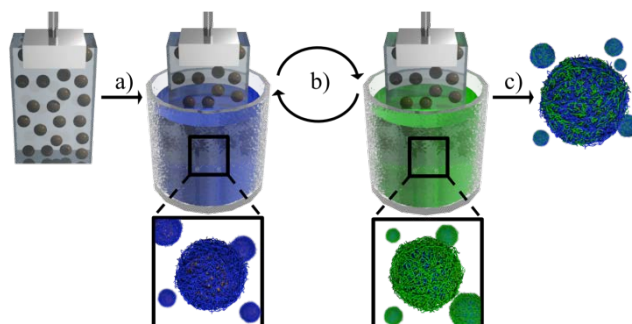
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Scheme 1. Template particle coating via IPA. a) Immobilized particles are immersed into a polymer solution, followed by washing steps (immersion in water). b) Subsequent immersion into the solution of a complementary polymer, followed by water washing steps. The water washing steps are not shown for simplicity. This process is repeated until the desired number of layers is attained. c) The layered particles are recovered from the agarose, and the template particles can be dissolved to yield capsules.

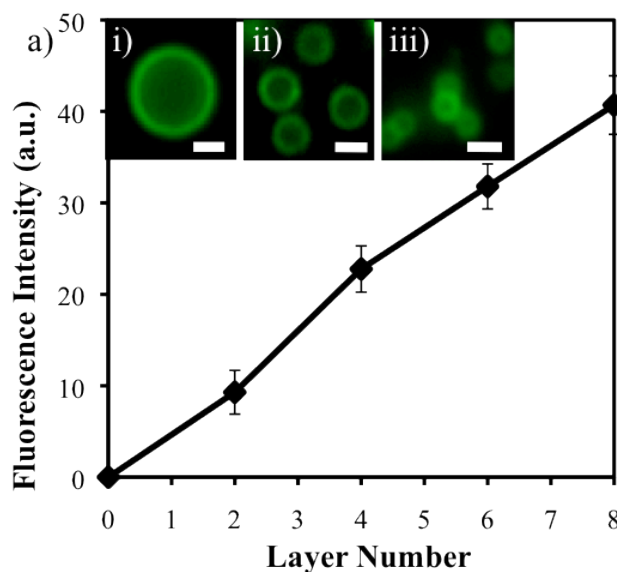


Figure 1. IPA layer buildup of four PAH/PSS multilayers and subsequent capsule preparation. Fluorescence intensity buildup of PAH-FITC/PSS on 2.59 μm -diameter SiO_2 particles. Odd layer numbers correspond to PAH layers and even layer numbers to PSS. Inset: Fluorescence microscopy images of capsules prepared on SiO_2 particles of diameter i) 2.59 μm , ii) 1.11 μm , and iii) 585 nm. All scale bars are 1 μm .

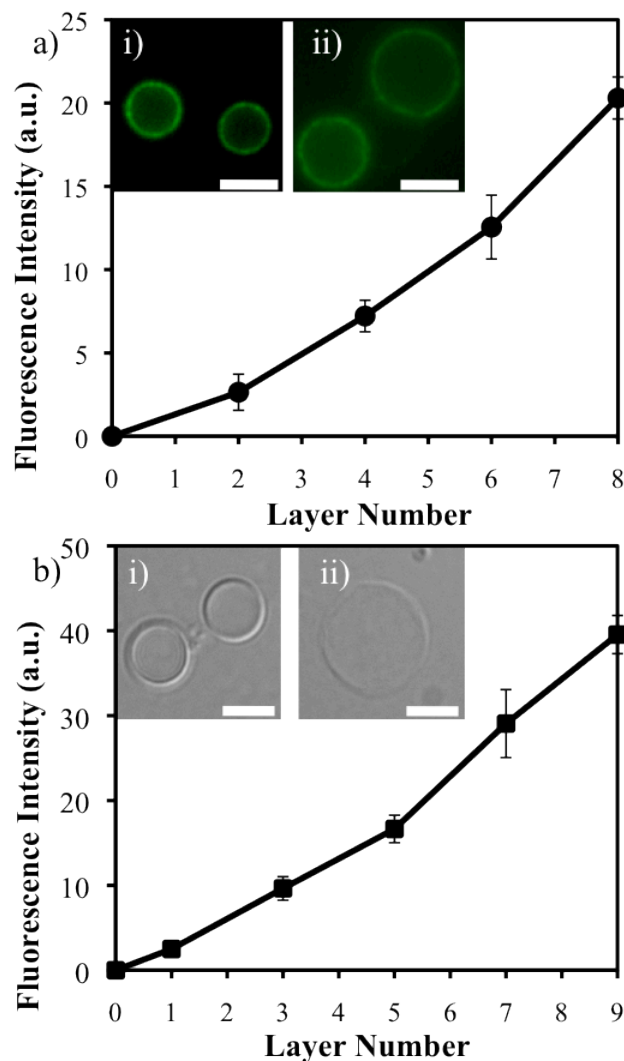


Figure 2. IPA layer buildup of PDPA_{AIK}/PMA and PVP/PMA_{SH} multilayers, and subsequent pH-responsive capsule preparation. a) Fluorescence intensity buildup of PDPA_{AIK}-AF488/PMA on 2.59 μm-diameter SiO₂ particles. Odd layer numbers correspond to PDPA_{AIK} layers and even layer numbers to PMA. Inset: Fluorescence microscopy images of i) PDPA_{AIK} capsules prepared from 2.59 μm-diameter SiO₂ particles at pH 4 and ii) pH 7.4. b) Fluorescence intensity buildup of PVP-AF488/PMA_{SH} on 2.59 μm-diameter SiO₂ particles. Odd layer numbers correspond to PVP-AF488 layers and even layer numbers to PMA_{SH}. Inset: DIC images of PMA_{SH} capsules prepared on 4.99 μm-diameter SiO₂ particles at i) pH 4 and ii) pH 7.4. All scale bars are 5 μm.

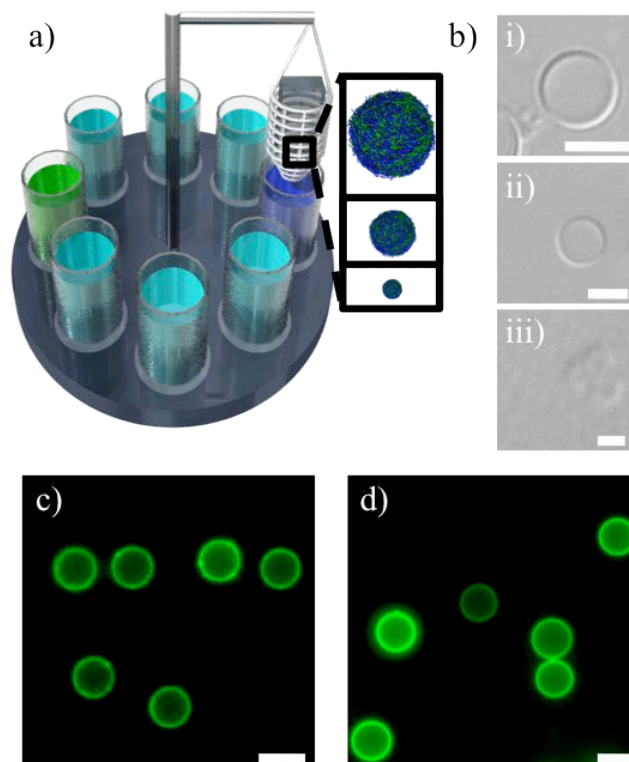


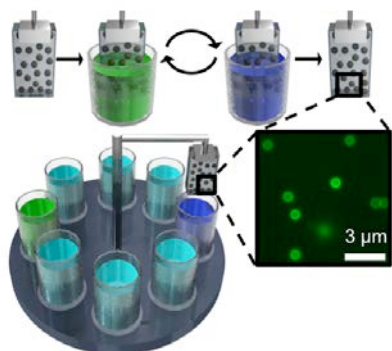
Figure 3. Capsules prepared via automated IPA. a) Schematic of the automated instrument. b) Four layer PMA_{SH} capsules at pH 4, prepared on SiO₂ particles of diameters i) 4.99 μm , ii) 2.59 μm , and iii) 585 nm. The scale bars are 5 μm , 3 μm , and 600 nm, respectively. c) Fluorescence microscopy image of four bilayer PAH/PSS capsules prepared on 2.59 μm -diameter SiO₂ particles and d) four layer PMA_{SH} capsules prepared on 4.99 μm -diameter SiO₂ particles at pH 4. The scale bars are 3 μm and 5 μm , respectively.

Keywords: immersive polymer assembly, colloids, layer-by-layer, polymer capsules, automated layering

Joseph J. Richardson, Kang Liang, Kristian Kempe, Hirotaka Ejima, Jiwei Cui, and Frank Caruso*

Immersive Polymer Assembly on Immobilized Particles for Automated Capsule Preparation

ToC entry



We report a versatile approach for polymer capsule preparation using immobilized particles, which are immersed into polymer solutions either manually or by using an automated robotic dipping machine. This technique produces polyelectrolyte capsules with improved retention over conventionally prepared capsules. Additionally, responsive hydrogel capsules of different diameter can be prepared simultaneously.