2-14-2011

Fabrication of Covalently Crosslinked and Amine-Reactive Microcapsules by Reactive Layer-by-Layer Assembly of Azlactone-Containing Polymer Multilayers on Sacrificial Microparticle templates

Eric M. Saurer
University of Wisconsin-Madison

Ryan M. Flessner
University of Wisconsin-Madison

Maren E. Buck
University of Wisconsin-Madison, mbuck@smith.edu

David M. Lynn
University of Wisconsin-Madison

Published in final edited form as:
J Mater Chem. 2011 February 14; 21(6): 1736–1745. doi:10.1039/C0JM02633F.

Fabrication of Covalently Crosslinked and Amine-Reactive Microcapsules by Reactive Layer-by-Layer Assembly of Azlactone-Containing Polymer Multilayers on Sacrificial Microparticle Templates

Eric M. Saurer[a,1], **Ryan M. Flessner**[a,1], **Maren E. Buck**[b], and **David M. Lynn**[a,b,*]

[a] Department of Chemical and Biological Engineering, University of Wisconsin – Madison, 1415 Engineering Drive, Madison, WI 53706, USA

[b] Department of Chemistry, University of Wisconsin – Madison, 1415 Engineering Drive, Madison, WI 53706, USA

Abstract

We report on the fabrication of covalently crosslinked and amine-reactive hollow microcapsules using 'reactive' layer-by-layer assembly to deposit thin polymer films on sacrificial microparticle templates. Our approach is based on the alternating deposition of layers of a synthetic polyamine and a polymer containing reactive azlactone functionality. Multilayered films composed of branched poly(ethylene imine) (BPEI) and poly(2-vinyl-4,4-dimethylazlactone) (PVDMA) were fabricated layer-by-layer on the surfaces of calcium carbonate and glass microparticle templates. After fabrication, these films contained residual azlactone functionality that was accessible for reaction with amine-containing molecules. Dissolution of the calcium carbonate or glass cores using aqueous ethylenediamine tetraacetic acid (EDTA) or hydrofluoric acid (HF), respectively, led to the formation of hollow polymer microcapsules. These microcapsules were robust enough to encapsulate and retain a model macromolecule (FITC-dextran) and were stable for at least 22 hours in high ionic strength environments, in low and high pH solutions, and in several common organic solvents. Significant differences in the behaviors of capsules fabricated on $CaCO_3$ and glass cores were observed and characterized using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Whereas capsules fabricated on $CaCO_3$ templates collapsed upon drying, capsules fabricated on glass templates remained rigid and spherical. Characterization using EDS suggested that this latter behavior results, at least in part, from the presence of insoluble metal fluoride salts that are trapped or precipitate within the walls of capsules after etching of the glass cores using HF. Our results demonstrate that the assembly of BPEI/PVDMA films on sacrificial templates can be used to fabricate reactive microcapsules of potential use in a wide range of fields, including catalysis, drug and gene delivery, imaging, and biomedical research.

Introduction

Layer-by-layer assembly is a versatile approach to the fabrication of nanostructured thin films and is typically accomplished by alternating the adsorption of 'mutually interacting' polymers on surfaces.[1] This approach has been used to design films with applications in catalysis, nanofiltration, the preparation of superhydrophobic or antimicrobial surface

[1]These authors contributed equally to this work.

coatings, drug and gene delivery, coatings for biomedical devices, and a wide range of other fields.[2] The versatile nature of layer-by-layer assembly permits the fabrication of films that conformally coat planar macroscopic surfaces as well as surfaces with complex geometries and microscale features, including micro- and nanoparticles. Multilayered films fabricated on micrometer-scale colloidal substrates have been used to design particles for gene and drug delivery, to protect proteins from enzymatic degradation, or to prepare microreactors and design hollow microcapsules.[3] As a general approach for the encapsulation of both large and small molecules and other agents, layer-by-layer assembly is attractive because the stability, thickness and permeability of the resulting capsule walls can be controlled and tuned by selecting appropriate polymers and conditions for the fabrication of the polymer films.[3a]

In this paper, we report an approach to the fabrication of covalently crosslinked and amine-reactive microcapsules that involves the layer-by-layer assembly of 'mutually reactive' polymers. Many methods for layer-by-layer assembly involve the alternating adsorption of oppositely charged polyelectrolytes to surfaces to fabricate 'polyelectrolyte multilayers' (or PEMs) held together by electrostatic interactions between adjacent polyelectrolyte layers.[1–2, 2d, 3a] However, layer-by-layer fabrication has also been accomplished by exploiting other interactions, such as hydrogen bonding[4] or covalent bonding,[5] between polymers containing complementary functional groups. Of particular relevance to the work described here, several groups have recently demonstrated the use of 'click'-type reactions between azide- and alkyne-functionalized polymers to fabricate films on sacrificial microparticle cores.[6] The subsequent removal of the core leads to hollow polymer microcapsules that are stable in a wider range of environmental conditions than microcapsules fabricated by electrostatic or hydrogen bonding layer-by-layer assembly.[4a, 4b, 6a, 7] In addition, the presence of residual reactive functional groups in the film after fabrication allows the capsules to be functionalized with a wide range of compounds to create capsules with desired properties (e.g., prevention of non-specific protein adsorption by functionalizing the surfaces of capsules with polyethylene glycol).[6c, 8]

We recently reported a method for the 'reactive' assembly of covalently crosslinked multilayered films using the azlactone-functionalized polymer poly(2-vinyl-4,4-dimethylazlactone) (PVDMA).[9] The highly strained azlactone rings in PVDMA (see Figure 1A) undergo rapid ring-opening reactions with a variety of nucleophiles, including primary amines, alcohols and thiols.[10] Our past studies demonstrated that ultrathin multilayered films can be fabricated by the layer-by-layer assembly of PVDMA with branched poly(ethylene imine) (BPEI), and that the primary amines of BPEI react readily with the azlactone rings of PVDMA in the absence of a catalyst. This process leads to the formation of diamide crosslinks (e.g., Figure 1A) between layers of the film without producing any byproducts.[9a] We have demonstrated that films fabricated using this approach contain residual azlactone functionality that is accessible for post-fabrication functionalization with primary amine-containing molecules, and that this approach can be used to prepare films with specific surface properties. For example, BPEI/PVDMA films treated with D-glucamine have been demonstrated to resist cell and protein adhesion for up to one month *in vitro*.[9b] We have demonstrated the fabrication of BPEI/PVDMA films on a variety of planar substrates,[9a, 9b] at interfaces between immiscible organic and aqueous phases,[9d] and on substrates with micron-scale topographical features.[9a, 9c]

This current study sought to investigate whether this approach could be used to fabricate reactive hollow microcapsules by fabricating BPEI/PVDMA films on sacrificial microparticle templates. This approach has several unique features that make it a potentially attractive alternative to other layer-by-layer approaches for creating reactive microcapsules. For example, compared to approaches based on the alkyne-azide Huisgen cycloaddition

reaction,[11] this approach does not require a catalyst to achieve rapid reactions at room temperature.[10] In addition, PVDMA is readily synthesized using conventional and living polymerization techniques,[10, 12] and no additional preparation steps are required after polymerization to render PVDMA reactive. Finally, the fabrication of BPEI/PVDMA films is conducted entirely in organic solvents, and could therefore potentially be used to coat the surfaces of water soluble templates that are not traditionally suitable as substrates for the aqueous-based layer-by-layer assembly of polyelectrolyte-based films.

The work reported here is described in two parts. In the first part, we describe the fabrication of multilayered BPEI/PVDMA films on calcium carbonate microparticles, and we demonstrate the ability to functionalize the resulting films with amine-functionalized molecules. Dissolution of $CaCO_3$ cores using aqueous EDTA resulted in the formation of flexible, hollow microcapsules that were stable in a wide range of aqueous and organic solutions. In the second part, we describe the fabrication of rigid, hollow microcapsules using glass microparticle templates that were etched away by treatment using hydrofluoric acid. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to characterize significant differences in the behaviors and physical properties of BPEI/PVDMA microcapsules fabricated using $CaCO_3$ and glass templates. Our results suggest a general and straightforward approach to the fabrication of reactive and hollow polymer microcapsules with potential applications in catalysis, drug delivery, imaging, and other areas of fundamental research.

Materials and Methods

Materials

Branched poly(ethylene imine) (MW = 25,000) and reagent grade acetone were obtained from Aldrich Chemical Company (Milwaukee, WI). 2-Vinyl-4,4-dimethylazlactone was a kind gift from Dr. Steven M. Heilmann (3M Corporation, Minneapolis, MN). Calcium chloride and sodium carbonate were purchased from Fisher Scientific (Pittsburgh, PA). Soda-lime glass beads (10–30 μm) were obtained from Polysciences Inc. (Warrington, PA). Ethylenediaminetetraacetic acid (EDTA) was purchased from Acros Organics (Geel, Belgium). Hydrofluoric acid (HF), reagent grade dimethyl sulfoxide (DMSO), and FITC-dextran (MW = 2,000,000) were obtained from Sigma-Aldrich (St. Louis, MO). Tetramethylrhodamine cadaverine (TMR-cadaverine) was purchased from Invitrogen Corporation (Carlsbad, CA). Poly(2-vinyl-4,4-dimethylazlactone) (PVDMA, MW = 18,000 – 25,000) was synthesized as previously described.[9c, 12a, 13] All materials were used as received without any additional purification unless noted otherwise.

Fabrication of Calcium Carbonate Particles

Calcium carbonate microparticles were fabricated using the approach described by DeGeest et al.[14] Briefly, aqueous solutions of calcium chloride and sodium carbonate were prepared at 0.33 M. Equal volumes of these solutions were mixed rapidly under vigorous stirring for 1 minute. Mixing led to rapid precipitation of $CaCO_3$ microparticles with diameters ranging from 3–6 μm. The $CaCO_3$ suspension was then centrifuged at full speed in a Centrific centrifuge (Fisher Scientific) for 5 minutes. The supernatant was decanted and the particles were rinsed in water to remove residual ions. The particles were centrifuged and rinsed in water four times. After decanting the supernatant from the fourth rinse in water, the particles were rinsed twice in acetone and centrifuged to remove water. The supernatant from the second rinse in acetone was decanted and the particles were dried under a stream of filtered air. Fabrication of $CaCO_3$ microparticles encapsulating FITC-dextran was accomplished by dissolving 5 mg FITC-dextran in 3.5 mL of 0.33 M calcium chloride prior to mixing the

calcium chloride with sodium carbonate, but was otherwise identical to the procedure described above.

Fabrication of Multilayered Films on Calcium Carbonate Particles and Glass Beads

Solutions of BPEI and PVDMA were prepared in acetone (40 mM with respect to the molecular weight of the polymer repeat unit). Approximately 3 mg of calcium carbonate particles were weighed out into microcentrifuge tubes and suspended in 250 μL of acetone. The first layer of BPEI was deposited onto the calcium carbonate particles by adding 250 μL of the BPEI solution (40 mM) to the particle suspension and vortexing the particles for 5 minutes to allow sufficient time for the polymer to adsorb to the particle surface. The particles were then centrifuged for 5 minutes at 1000 g. The supernatant was removed by pipette and the particles were rinsed three times by resuspending them in 500 μL of acetone and vortexing. After each rinse in acetone, the particles were centrifuged for 5 minutes at 1000 g and the supernatant was removed. After the third acetone rinse, the particles were resuspended in 250 μL of acetone and transferred by pipette to a new microcentrifuge tube. The second layer of the multilayered film was fabricated by adding 250 μL of the PVDMA solution (40 mM) to the particle suspension and vortexing for 1 minute to allow sufficient time for the PVDMA to react to the previously deposited BPEI layer. The particles were then rinsed three times with 500 μL of acetone and transferred to a new microcentrifuge tube in 250 μL of acetone as described above. Subsequent layers were fabricated by repeating this process, alternately adding BPEI or PVDMA layers and allowing each layer to react for 1 minute, until the desired number of polymer BPEI/PVDMA layer pairs (or ‘bilayers’; typically 5) were deposited onto the particle surface. After multilayered film fabrication, the particles were stored in acetone overnight until further use. The coating of glass beads was performed following the procedure above with the following exceptions: 1) glass beads were suspended in 700 μL of acetone, 2) 700 μL of polymer solution was added during each of the deposition steps, 3) glass beads were rinsed in 1 mL of acetone, and 4) glass beads were not transferred to new microcentrifuge tubes between layers to avoid the loss of glass beads. The morphology of the coated $CaCO_3$ or glass microparticles was characterized using scanning electron microscopy (SEM) as described below.

Post-Fabrication Functionalization of Coated Particles

To characterize the presence and accessibility of residual azlactone groups contained in the BPEI/PVDMA films, the films were functionalized using a variety of primary amine-containing molecules. For example, the coated particles were functionalized with TMR-cadaverine after film fabrication to permit visualization of the film using fluorescence and laser scanning confocal microscopy. For these experiments, calcium carbonate particles and glass beads coated with BPEI/PVDMA films were centrifuged at 1000 g. The acetone supernatant was decanted by pipette and the coated particles were resuspended in 200 μL of DMSO containing 0.5 mg/mL TMR-cadaverine. The particles were placed on a microcentrifuge tube rotator and allowed to react with the TMR-cadaverine for at least 3 hours. After functionalization with TMR-cadaverine, the particles were centrifuged at 1000 g and the TMR-cadaverine solution was decanted. The particles were then rinsed in DMSO followed by a rinse in deionized water. The particles were then resuspended in acetone until further use. The same general procedure was used for functionalizing the film-coated particles with propylamine (50 mM in DMSO), except this reaction was allowed to proceed overnight. Coated microparticles that were functionalized with TMR-cadaverine or propylamine were characterized using fluorescence and laser-scanning confocal microscopy as described below.

Preparation of Hollow Multilayered Polymer Capsules

Experiments designed to fabricate hollow multilayered polymer shells were conducted by first coating calcium carbonate microparticles with BPEI/PVDMA films 5 bilayers thick. The calcium carbonate cores were then dissolved using the following protocol. Coated $CaCO_3$ particles were suspended twice in 500 μL of aqueous EDTA (0.2 M, pH = 5) at room temperature for 30 minutes. After each incubation in EDTA solution, the particles/capsules were centrifuged at 9000 g for 5 minutes and the supernatant was decanted by pipette. The capsules were then briefly rinsed in 500 μL of EDTA solution, centrifuged (9000 g for 5 minutes), rinsed briefly in 500 μL deionized water, centrifuged, and resuspended in 250 μL acetone. The integrity and morphology of the hollow capsules were evaluated using fluorescence, confocal, and scanning electron microscopy (SEM) as described below.

Hollow multilayered polymer capsules were obtained from BPEI/PVDMA films coated on glass beads by etching out the glass core using the following procedure. BPEI/PVDMA coated glass beads were incubated in 1 mL of 10% hydrofluoric acid (HF) at room temperature for approximately 24 hours. [WARNING: HF solutions and vapors are extremely poisonous and corrosive, and may cause extreme burns that are not immediately painful! Handle with extreme caution, in a chemical fume hood, and using appropriate protective equipment (gloves, face/eye protection, lab coat, etc.), and neutralize waste appropriately. Do not store in glass containers.] The HF solution was refreshed twice during the etching process (after 7 and 22 hours) by centrifuging the capsules at 9000 g for 5 minutes, removing the supernatant by pipette, and resuspending the capsules in fresh 10% HF solution. After 24 hours, the capsules were centrifuged (9000 g for 5 minutes) and rinsed three times in 1 mL of deionized water. After the third rinse cycle, the capsules were resuspended in 500 μL of acetone. The morphology of the hollow capsules was characterized using confocal and scanning electron microscopy as described below.

For experiments designed to evaluate the effect of HF treatment on hollow capsules prepared using $CaCO_3$ cores (see text), hollow capsules were prepared as described above. After the final rinse in water, the capsules were resuspended in 795 μL of deionized water. For samples treated with HF in the presence of uncoated glass microspheres, ~3 mg of glass microspheres were added to the suspension. Then, 205 μL of 48 wt% HF stock solution was added to these suspensions to yield a final concentration of 10 wt% HF. The HF solution was refreshed periodically as described above for capsules prepared on glass particles, by centrifuging the suspension (9000 g for 5 minutes), removing the supernatant, resuspending the capsules in 795 μL deionized water and adding 205 μL of 48 wt% HF. After 24 hours of incubation in HF, the capsules were rinsed as described above for capsules prepared on glass particles and resuspended in 250 μL acetone. The morphology of the hollow capsules after HF treatment was evaluated using SEM as described below.

Characterization of Microcapsule Stability

Experiments designed to evaluate the stability of BPEI/PVDMA microcapsules in high and low pH solutions, high ionic strength solutions, and various organic solvents were conducted as follows. Hollow microcapsules were prepared using $CaCO_3$ cores as described above. The capsules were centrifuged at 9000 g for 5 minutes, and the acetone was removed by pipette. The capsules were resuspended in 250 μL of 5 M hydrochloric acid, 5 M sodium hydroxide, saturated aqueous sodium chloride, acetone, dichloromethane, or methanol. The suspensions were rotated end-over-end on a mechanical laboratory rotator (Barnstead Thermolyne, Dubuque, IA) for 22 hours at ambient room temperature. For capsules in 5 M HCl, 5 M NaOH, or saturated NaCl, the suspensions were centrifuged (9000 g for 5 minutes), the supernatant was removed by pipette, and the capsules were rinsed in 500 μL deionized water. The capsules were then centrifuged and resuspended in 250 μL deionized

water. Capsules suspended in organic solvents were not rinsed. Capsule integrity was evaluated using fluorescence microscopy as described below.

Fluorescence and Laser-Scanning Confocal Microscopy

Samples of coated microparticles or hollow microcapsules for fluorescence and confocal microscopy were prepared from particle or capsule suspensions in acetone, dichloromethane, methanol or water. All fluorescence and confocal microscopy images were acquired with the particles suspended in water. For samples initially suspended in organic solvents, a 10 μL aliquot of the suspension was placed on a glass microscope slide and the solvent was allowed to evaporate. A 10 μL drop of deionized water was then placed on the microscope slide and covered with a glass cover slip. For samples initially suspended in water, a 10 μL aliquot of the suspension was placed on a glass microscope slide and covered with a glass cover slip. Fluorescence microscopy images were obtained using an Olympus IX70 microscope and analyzed using a Metavue (v. 7.1.2.0) software package (Universal Imaging Corporation). Laser-scanning confocal microscopy (LSCM) was performed using a Bio-Rad Radiance 2100 MP Rainbow laser-scanning confocal microscope. The confocal images were acquired using the Bio-Rad LaserSharp 2000 software package. Adobe Photoshop and ImageJ (National Institutes of Health, Bethesda, MD) were used to further process both the fluorescence and confocal images.

Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy

Samples of uncoated or coated microparticles and hollow microcapsules for scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were prepared from particle or capsule suspensions in acetone. A 10 μL aliquot of a particle or capsule suspension was placed in the center of an aluminum SEM sample holder (Ted Pella, Inc.) and the acetone was allowed to evaporate. The samples were stored in a vacuum dessicator for at least 1 hour. Prior to SEM imaging and EDS analysis the samples were coated with a thin layer of gold using a Desk II sputterer (Denton Vacuum, Moorestown, NJ; 30 s at 45 mA, 50 mTorr). SEM was conducted using a LEO DSM 1530 scanning electron microscope at an accelerating voltage of 3kV. EDS was conducted on the same instrument using an UltraDry X-ray detector (ThermoFisher Scientific) at an accelerating voltage of 8 kV. Spectroscopy data were analyzed using the NMSS software package.

Results and Discussion

Fabrication of Hollow Microcapsules by Reactive Layer-by-Layer Assembly on $CaCO_3$ Cores

Layer-by-layer assembly has been used to fabricate microcapsules for a broad range of applications.[3a, 15] In past studies, these microcapsules were fabricated on sacrificial templates by the alternate and repetitive deposition of mutually interacting polymers, followed by dissolution of the template.[3a] The properties of the resulting microcapsules generally depend upon several factors, including the type of molecular interactions used for film assembly (e.g., electrostatic interactions, hydrogen bonding, disulfide bonding, or covalent bonding), the structures of the polymers that are incorporated in the films, the thicknesses of the polymer films, the solution conditions used for film fabrication, and the conditions to which the capsules are exposed after fabrication. The details of film assembly and film properties of microcapsules fabricated in a broad range of environments have been reviewed comprehensively.[3a, 15h, 16]

In this study, we sought to determine whether BPEI/PVDMA multilayered films could be fabricated on sacrificial colloidal templates and whether this approach would yield reactive, covalently crosslinked, and highly stable microcapsules (Figure 1B). We selected calcium

carbonate microparticles as the sacrificial core for fabricating reactive microcapsules in our initial studies for several reasons. First, calcium carbonate microparticles with diameters ranging from ~3–6 μm can be readily prepared by precipitation from solutions of calcium chloride and sodium carbonate.[17] Unlike many polymer microparticles used previously as sacrificial cores for the fabrication of hollow microcapsules, $CaCO_3$ cores are insoluble and do not swell in the organic solvents used for fabrication of the BPEI/PVDMA multilayered films described here. In addition, these calcium carbonate particles are porous, and can be loaded with macromolecular cargoes prior to fabrication of films by soaking the microparticles in solutions of a macromolecule of interest[17] or by co-precipitation[14, 18] of a macromolecule. Finally, calcium carbonate cores can be dissolved in mild, aqueous EDTA solutions, and the Ca^{2+} and CO_3^{2-} ions have been demonstrated to diffuse readily through the walls of multilayered microcapsules.[14] In this study, calcium carbonate microparticles loaded with FITC-dextran were prepared using a co-precipitation protocol previously described by DeGeest, et al. (see Materials and Methods for additional details).[14] Figure 2A shows a representative SEM image of a $CaCO_3$ microparticle prepared using this method. Inspection of this image reveals that the roughly spherical microparticle has a diameter of ~4.5 μm and that it is an amorphous particle composed of smaller (~50–100 nm) $CaCO_3$ nanoparticles.[14]

As the first step toward the fabrication of hollow microcapsules, covalent layer-by-layer assembly was used to coat the surfaces of $CaCO_3$ microparticles with BPEI/PVDMA films (see Figure 1). Layers of BPEI and PVDMA were deposited on the $CaCO_3$ cores by iterative and successive cycles of particle suspension, centrifugation, and resuspension in solutions of BPEI or PVDMA. To allow adsorption of the first layer of polymer on the surfaces of the microparticles, the $CaCO_3$ microparticles were suspended in a solution of BPEI for 5 minutes. All subsequent layers were allowed to react for 1 minute. Past studies have demonstrated that one minute reaction times are sufficient for the reaction of the primary amines on BPEI with the azlactone groups of PVDMA during layer-by-layer assembly on planar substrates.[9a] Figure 2B shows a representative SEM image of a $CaCO_3$ microparticle coated with a BPEI/PVDMA film 5 layer pairs (or ‘bilayers’) thick. The surface of the film appears uniformly smooth, and no large-scale defects are observed in the film by SEM. The film coating on the $CaCO_3$ template masks, to some extent, the appearance of the nanoparticles observed in Figure 2A. While it is experimentally difficult to measure the thickness of films fabricated on $CaCO_3$ microparticles directly, our past studies of films fabricated on planar, silicon substrates demonstrate that BPEI/PVDMA films 5 bilayer thick are approximately 30–60 nm thick.[9a] We return to a consideration of film thickness again in the discussion below.

We have demonstrated in past studies that BPEI/PVDMA films contain residual azlactone functionality that is accessible for reaction with amine-containing molecules, including tetramethylrhodamine cadaverine (TMR-cadaverine; a fluorescent molecule) and D-glucamine (a molecule used to prevent cell and protein adhesion on surfaces).[9] To further confirm the presence of BPEI/PVDMA films on the surfaces of the microparticles described above, we suspended the coated particles in solutions of TMR-cadaverine. Figure 2C shows a representative confocal microscopy image of coated particles with films that were functionalized with TMR-cadaverine. The green fluorescence observed in the core of the particles indicates the presence of FITC-dextran encapsulated in the $CaCO_3$ particles, and the ring of red fluorescence around each particle arises from the presence of TMR-cadeverine. The particles appear uniformly coated, and no defects or uncoated areas were observed in these films. Coated particles that were first reacted with propylamine (to consume any residual azlactone functionality)[9a] before incubating the films with TMR-cadaverine did not show evidence of red fluorescence on the particle surface (data not shown). This result suggests that the red fluorescence observed in Figure 2C results from the

covalent attachment of TMR-cadaverine to azlactone groups in the film and not from the physical adsorption of TMR. This observation is consistent with our previous studies of BPEI/PVDMA films on planar macroscopic substrates and natural and synthetic fibers.[9a, 9c]

In addition to single, isolated particles, Figure 2C reveals the presence of several small aggregates of up to 8 microparticles. We note that these aggregates could be formed during the fabrication of the films (e.g., due to reactions between BPEI and PVDMA on adjacent particles during layer-by-layer assembly) or could result from suspension of the relatively hydrophobic coated particles in water for imaging. Further optimization of the fabrication process (to minimize reactions between particles) or functionalization of the coated particles with more hydrophilic moieties (e.g., glucamine, PEG-amine) could help to reduce such aggregation. However, the methods described above yielded suspensions of coated particles sufficient for all characterization studies described below.

To determine whether the BPEI/PVDMA films described above could withstand removal of the $CaCO_3$ cores, we treated film-coated particles with aqueous EDTA (0.2 M, pH = 5.0). The particles were twice suspended in EDTA solution for 30 minutes at room temperature, then rinsed with EDTA solution and deionized water (see Materials & Methods for additional details). The resulting capsules were resuspended in acetone for further characterization. Figure 2D shows a representative SEM image of a hollow BPEI/PVDMA microcapsule. When dried for characterization by SEM, the microcapsules collapsed and flattened on the surface of the SEM sample stage, similar to examples in past reports on the characterization of hollow, multilayered microcapsules.[3a] By measuring the thicknesses of vertical folds in these collapsed capsules (see Figure 2D), we estimated the walls of these capsules (composed of 5 bilayers of BPEI/PVDMA) to be approximately 100 nm thick. This value is somewhat thicker than the thicknesses of films fabricated with an identical number of layers on planar, silicon substrates (e.g., 30–60 nm).[9a] This increased thickness could result from differences in the chemical composition and physical properties (e.g., porosity) of the $CaCO_3$ particles compared to silicon and glass substrates, or other factors related to the fabrication of films on $CaCO_3$ microparticles or post-fabrication treatment to remove the cores. While ripped or otherwise damaged microcapsules were occasionally observed, the vast majority of the capsules appeared to remain intact and unbroken. The surfaces of the microcapsules were similar in appearance to the surfaces of the films before dissolution of the $CaCO_3$ cores, suggesting that treatment with EDTA did not substantially change the morphology or general structure of the films.

We also used fluorescence and confocal microscopy to characterize fluorescently labeled microcapsules (prepared by reacting the BPEI/PVDMA films with TMR-cadaverine prior to dissolving the $CaCO_3$ cores) after removal of the $CaCO_3$ cores. For these experiments, a 10 μL aliquot of the capsule suspension was placed on a microscope slide, and the acetone was allowed to evaporate. The dried microcapsules appeared collapsed and flattened (data not shown), similar to the capsules imaged by SEM (as shown in Figure 2D). Upon rehydration with deionized water, however, the microcapsules returned to a spherical shape. Figure 2F shows a representative confocal microscopy image of the spherical, fluorescently labeled microcapsules. The presence of bright green fluorescence in the centers of the capsules demonstrates the ability of these BPEI/PVDMA capsules to encapsulate and retain FITC-dextran after removal of the $CaCO_3$ cores. Red fluorescence in the capsule shell results from TMR-cadaverine that was reacted with the film before dissolution of the core. As described above for SEM, a small number of broken capsules (identified by large defects in the capsule wall and substantially reduced levels of green fluorescence) were observed, but the vast majority of capsules remained intact throughout the fabrication and dissolution processes as well as subsequent rinsing and centrifugation steps. These results demonstrate

the ability of multilayered films fabricated using BPEI and PVDMA to withstand removal of sacrificial $CaCO_3$ cores to form hollow microcapsules.

One of the potential advantages of using covalently crosslinked multilayered assemblies to create polymer microcapsules (compared to electrostatically crosslinked or hydrogen-bonded films) is that the capsule shells have the potential to be stable over a wide range of environmental conditions. For example, depending on the materials selected for the assembly of polyelectrolyte-based capsules, exposing the capsules to changes in pH or ionic strength or certain organic solvents can disrupt the interactions that hold the film together and cause the capsules to disassemble or become permeable to large molecules.[3a, 4a, 4b, 6a, 7] To evaluate the stability of our BPEI/PVDMA capsules, we suspended hollow capsules (fabricated using $CaCO_3$ cores as described above) in saturated aqueous NaCl, 5 M HCl, or 5 M NaOH solutions at room temperature for 22 hours. After incubation, the capsules were rinsed twice and resuspended in deionized water. Figure 2E shows a representative fluorescence microscopy image of microcapsules incubated in saturated NaCl solution. The bright green fluorescence observed in the capsule demonstrates that FITC-dextran encapsulated by the BPEI/PVDMA films is retained within the capsules, and suggests that the capsules are stable for at least 22 hours in this high ionic strength solution. Fluorescence microscopy images of capsules that were incubated in 5 M HCl or 5 M NaOH solutions are shown in Figure S1 of the Supporting Information and demonstrate that the capsules are stable for at least 22 hours in both high and low pH solutions. We also evaluated the stability of these covalently crosslinked capsules in acetone, methanol, and dichloromethane (Figure S1). The capsules did not appear to be damaged by exposure to these organic solvents. We note that characterization of the capsules by fluorescence microscopy can be used to identify capsules that have not ruptured, but it does not permit direct imaging of capsules that may be disrupted or damaged by exposure to the conditions described above. However, capsules observed by phase contrast microscopy before and after these stability studies were also qualitatively similar, suggesting that substantial fractions of these capsules remain intact. The stability of the BPEI/PVDMA capsules suggests that this approach could be used to fabricate microcapsules for applications that require encapsulation of species in a wide range of environmental conditions.

Fabrication of Hollow Microcapsules on Glass Microspheres

To explore the range of sacrificial templates that could be used as substrates for the fabrication of BPEI/PVDMA microcapsules, we investigated the fabrication of BPEI/PVDMA films on soda-lime glass microspheres with diameters ranging from ~10 – 30 μm. The fabrication of the BPEI/PVDMA films on glass was performed in a similar manner to the approach described above for fabrication of BPEI/PVDMA films on $CaCO_3$. Figures 3A and 3B show representative SEM images of an uncoated glass microsphere and a glass microsphere coated with a BPEI/PVDMA film 5 bilayers thick, respectively. The surfaces of the uncoated glass particles were rough, with large (up to ~3 μm diameter) features that protruded from the surfaces of the particles. After coating the glass microspheres with BPEI/PVDMA films, these features appeared less well defined, suggesting that the polymer films were covering the surfaces of the particles uniformly.

To further evaluate the uniformity of the films, the coated glass particles were functionalized with TMR-cadaverine (as described above for $CaCO_3$ cores), and characterized using confocal microscopy. Figure 3C shows a representative confocal microscopy image of glass microspheres coated with TMR-functionalized BPEI/PVDMA films. The red fluorescence observed in the image is uniform around the circumference of the particles, and large-scale defects in the films are not observed. As described above, films that were functionalized with propylamine prior to reaction with TMR-cadaverine did not show evidence of red fluorescence, suggesting that the red fluorescence shown in Figure 3C is a result of

covalently bound TMR-cadaverine and not TMR-cadaverine that is physically adsorbed to or trapped within the film.

To investigate the ability of the BPEI/PVDMA films to withstand the etching of the glass templates, the polymer-coated glass microspheres were incubated in hydrofluoric acid (10 wt %) for approximately 24 hours. Figure 3D shows a representative SEM image of microcapsules after removal of the glass cores and reveals the presence of both intact and broken capsules. Interestingly, and in sharp contrast to the microcapsules fabricated on $CaCO_3$ templates described above, these microcapsules remained spherical and did not collapse when dried. Figure 3E shows the inside surface of a broken, hollow capsule shell. Broken capsules remained rigid when dried on the SEM stage, retaining a dome- or bowl-like shape rather than flattening or collapsing. Previous studies by Caruso et al. have demonstrated the formation of rigid, hollow microparticles by the layer-by-layer deposition of silica nanoparticles and PDADMAC on microparticle templates.[16, 19] However, to our knowledge, the fabrication of rigid capsules using layer-by-layer assembly of polymers (i.e., without the incorporation of nanoparticles or other non-polymeric additives) has not been reported previously. Figure 3F shows a representative confocal microscopy image of TMR-functionalized capsules after dissolution of the glass core. Red fluorescence is observed to be uniform around the capsules, although some small cracks or other defects in the films are visible. These cracks and defects are also apparent in the SEM image shown in Figure 3D. Despite the appearance of these imperfections and holes, the capsules appear to retain their spherical shapes, further supporting the conclusion that these capsules are more rigid than the BPEI/PVDMA capsules fabricated on $CaCO_3$ cores described above.

We conducted a series of experiments to investigate the factors that could contribute to the formation of the rigid capsules observed in Figure 3D–F. Inspection of the inset in Figure 3E (which shows a higher magnification view of the capsule shell shown in Figure 3E) reveals that the capsule shell is approximately 1 μm thick. This is substantially thicker than the films fabricated on $CaCO_3$ (as estimated above) and in our past reports of BPEI/PVDMA films on planar substrates.[9a] Initial examination of the inner surfaces of broken capsules by SEM (as shown in Figure 3E) also reveals the presence of small particles along the interior walls of the capsules. One possible explanation for the increase in the thicknesses of these films and the particles observed along the interior of the walls of the capsules is precipitation of insoluble compounds (such as CaF_2 or MgF_2 as discussed below) on or within the polymer shell during the etching of the glass particles. We used energy dispersive X-ray spectroscopy (EDS) to characterize the atomic compositions of the coated glass particles and hollow polymer shells formed by dissolution of the glass core (Table 1). EDS analysis of rigid, hollow capsules formed by treatment with HF showed significantly reduced silicon and oxygen content compared to coated glass particles (consistent with the dissolution of SiO_2 by HF), and identified the presence of fluorine, calcium, and magnesium in substantial quantities. In view of the fact that soda-lime glass is composed primarily of silicon dioxide, with lower amounts of sodium oxide, magnesium oxide, and calcium oxide,[20] these results suggest that calcium fluoride and magnesium fluoride formed during the etching of the glass core with HF could precipitate within walls of the BPEI/PVDMA capsules. Closer inspection of the EDS data (Table 1) demonstrates that the fluorine:(calcium + magnesium) ratio is approximately 2:1, which is consistent with the hypothesis that MgF_2 and CaF_2 are present in the rigid microcapsules.

To further test the hypothesis that the precipitates on the BPEI/PVDMA films after the dissolution of the glass core were metal fluoride salts, we treated the rigid, hollow capsules with aqueous solutions of EDTA (0.2 M), HCl (1 M), or deionized water for 2 hours. Calcium and magnesium ions are chelated by EDTA and calcium fluoride and magnesium fluoride are more soluble in acidic media than at neutral pH. After several rinses in water,

the particles were resuspended in acetone and imaged using SEM. The results of these experiments demonstrated that the rigid, hollow microcapsules became flexible and flattened when dried on the SEM stage following treatment with EDTA (Figure 4B) and HCl (not shown), whereas capsules that were soaked in water remained rigid. EDS analysis of the flattened microcapsules indicated a significant reduction in the calcium content of these samples (Table 1), but indicated that significant levels of magnesium and fluorine (~10, 18 atom %, respectively) were still present in the collapsed capsules. These results suggest that the metal fluoride salts contribute significantly to the rigidity of the hollow capsules, but that complete removal of magnesium, calcium, and fluorine may not be necessary to promote the transition to flexible microcapsules.

To determine the extent to which HF alone could have resulted in the formation of the rigid capsules described above, we incubated hollow capsules prepared using $CaCO_3$ templates in 10% HF for ~24 hours. We also performed similar experiments using suspensions of film-coated $CaCO_3$ templates to which uncoated glass particles were also added (to create environments that would, upon addition of HF, expose these films to the inorganic compounds generated by the etching of glass). SEM characterization of capsules treated with HF, both in the presence and absence of uncoated glass particles, demonstrated that the hollow capsules remained flexible and that they collapsed when dried on an SEM stage (Figures 4C and 4D). The image in Figure 4D shows particulate debris on the outside of the hollow capsules that could arise from insoluble precipitates formed by dissolution of uncoated glass particles by HF, but these precipitates do not appear to influence the rigidity of the hollow capsules. These results suggest that the formation of rigid hollow capsules using glass beads as templates is a consequence of the dissolution of the glass particles (and the formation of insoluble salts) from the interior of the polymer films during etching, and not treatment of the film with HF alone or the simple presence of inorganic byproducts of glass etching in solution.

Summary and Conclusions

We have demonstrated a layer-by-layer approach to the fabrication of covalently crosslinked and amine-reactive microcapsules by alternately depositing azlactone-functionalized PVDMA and BPEI onto sacrificial calcium carbonate and glass templates. Residual azlactone functionality present in the films was used to functionalize the capsules with a primary amine-containing fluorophore prior to dissolution of the core templates. The dissolution of the $CaCO_3$ or glass cores resulted in hollow microcapsules that were stable in high ionic strength environments, in solutions of both high and low pH, and in several organic solvents. Characterization of the particles after removal of the cores indicated that capsules fabricated on calcium carbonate were spherical when wet but collapsed when dried, whereas capsules fabricated on glass templates remained rigid after removal of the glass cores. Further characterization of these rigid particles suggested that the rigidity resulted, at least in part, from the precipitation of metal fluoride salts on the inside of the multilayered shell. Post-etching treatment of these rigid capsules with aqueous EDTA restored the flexibility of these capsules.

The work reported here describes a general approach for the fabrication of reactive, covalently crosslinked microcapsules that could be of interest in a broad range of fundamental and applied contexts. Our results demonstrate that high molecular weight FITC-dextran can be encapsulated in BPEI/PVDMA capsules and that it is retained within these capsules upon exposure to a wide range of solvents, ionic strengths, and values of pH. These results suggest opportunities to develop microcapsules that could be used to protect, transport, or control the release of large or small molecules or other agents encapsulated within the capsules. Our results also demonstrate that the presence of residual azlactone

groups in the BPEI/PVDMA films can be utilized to functionalize the surfaces of the capsules. These methods could be used to confer specific chemical or biological functionality to hollow microcapsules and tune their properties post-fabrication. When combined, our results provide the basis of approaches for the preparation of reactive, covalently crosslinked microcapsules for applications in catalysis, imaging, gene and drug delivery, and a wide range of other fields.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Alfred P. Sloan Foundation, the NSF (DMR-0520527) through a grant to the Materials Research Science and Engineering Center (MRSEC) at the University of Wisconsin, and the National Institutes of Health (1 R01 EB006820). We thank Dr. Steven Heilmann for providing the samples of 2-vinyl-4,4-dimethylazlactone (VDMA). E.M.S. was supported in part by a 3M Foundation Graduate Research Fellowship. M.E.B. was funded in part by an NIH Chemistry Biology Interface Training Grant (NIGMS T32 GM008505). We thank Lance Rodenkirch, Michael Hendrickson, and the W. M. Keck Center for Biological Imaging at UW for support, training, and access to the confocal microscopy facilities, and Rick Noll and the Materials Science Center at UW for training and access to SEM and EDS facilities. D.M.L. is an Alfred P. Sloan Research Fellow.

References

1. Decher G. Science 1997;277:1232–1237.
2. (a) Hammond PT. Adv Mater 2004;16:1271–1293. (b) Bertrand P, Jonas A, Laschewsky A, Legras R. Macromol Rapid Commun 2000;21:319–348. (c) Ai H, Jones S, Lvov Y. Cell Biochem Biophys 2003;39:23–43. [PubMed: 12835527] (d) Boudou T, Crouzier T, Ren KF, Blin G, Picart C. Adv Mater 2010;22:441–467. [PubMed: 20217734] (e) Jewell CM, Lynn DM. Adv Drug Deliv Rev 2008;60:979–999. [PubMed: 18395291]
3. (a) Peyratout CS, Dähne L. Angew Chem Int Ed 2004;43:3762–3783. (b) Angelatos AS, Katagiri K, Caruso F. Soft Matter 2006;2:18–23. (c) De Geest BG, Sanders NN, Sukhorukov GB, Demeester J, De Smedt SC. Chem Soc Rev 2007;36:636–649. [PubMed: 17387411]
4. (a) Kharlampieva E, Sukhishvili SA. Polym Rev 2006;46:377–395. (b) Sukhishvili SA, Granick S. J Am Chem Soc 2000;122:9550–9551. (c) Sukhishvili SA, Granick S. Macromolecules 2002;35:301–310. (d) Zhang YJ, Guan Y, Yang SG, Xu J, Han CC. Adv Mater 2003;15:832–835.
5. (a) Chen JY, Luo GB, Cao WX. Macromol Rapid Commun 2001;22:311–314. (b) Zhang YJ, Yang SG, Guan Y, Cao WX, Xu J. Macromolecules 2003;36:4238–4240. (c) Liang ZQ, Wang Q. Langmuir 2004;20:9600–9606. [PubMed: 15491192] (d) Such GK, Quinn JF, Quinn A, Tjipto E, Caruso F. J Am Chem Soc 2006;128:9318–9319. [PubMed: 16848452] (e) Liang ZQ, Dzienis KL, Xu J, Wang Q. Adv Funct Mater 2006;16:542–548. (f) Rydzek G, Thomann JS, Ben Ameur N, Jierry L, Mesini P, Ponche A, Contal C, El Haitami AE, Voegel JC, Senger B, Schaaf P, Frisch B, Boulmedais F. Langmuir 2010;26:2816–2824. [PubMed: 19950954]
6. (a) Such GK, Tjipto E, Postma A, Johnston APR, Caruso F. Nano Lett 2007;7:1706–1710. [PubMed: 17530811] (b) De Geest BG, Van Camp W, Du Prez FE, De Smedt SC, Demeester J, Hennink WE. Macromol Rapid Commun 2008;29:1111–1118. (c) Ochs CJ, Such GK, Städler B, Caruso F. Biomacromolecules 2008;9:3389–3396. [PubMed: 18991459] (d) Cavalieri F, Postma A, Lee L, Caruso F. ACS Nano 2009;3:234–240. [PubMed: 19206271] (e) Huang, C-j; Chang, F-c. Macromolecules 2009;42:5155–5166.
7. Quinn JF, Johnston APR, Such GK, Zelikin AN, Caruso F. Chem Soc Rev 2007;36:707–718. [PubMed: 17471396]
8. Kinnane CR, Wark K, Such GK, Johnston AP, Caruso F. Small 2009;5:444–448. [PubMed: 19173351]
9. (a) Buck ME, Zhang J, Lynn DM. Adv Mater 2007;19:3951–3955. (b) Buck ME, Breitbach AS, Belgrade SK, Blackwell HE, Lynn DM. Biomacromolecules 2009;10:1564–1574. [PubMed:

19438231] (c) Buck ME, Lynn DM. ACS Appl Mater Interfaces 2010:1421–1429. [PubMed: 20402471] (d) Buck ME, Lynn DM. Adv Mater 2010;22:994–998. [PubMed: 20217827]

10. Heilmann SM, Rasmussen JK, Krepski LR. J Polym Sci A Polym Chem 2001;39:3655–3677.
11. Kolb HC, Finn MG, Sharpless KB. Angew Chem Int Ed 2001;40:2004–2021.
12. (a) Guichard B, Noel C, Reyx D, Thomas M, Chevalier S, Senet JP. Macromol Chem Phys 1998;199:1657–1674. (b) Fournier D, Pascual S, Fontaine L. Macromolecules 2004;37:330–335. (c) Lokitz BS, Messman JM, Hinestrosa JP, Alonzo J, Verduzco R, Brown RH, Osa M, Ankner JF, Kilbey SM. Macromolecules 2009;42:9018–9026. (d) Tully DC, Roberts MJ, Geierstanger BH, Grubbs RB. Macromolecules 2003;36:4302–4308.(e) Schilli, CM.; Muller, AHE.; Rizzardo, E.; Thang, SH.; Chong, YK. Advances in Controlled/Living Radical Polymerization. Matyjaszewski, K., editor. Vol. 854. Amer Chemical Soc; Washington: 2003. p. 603-618. (f) Messman JM, Lokitz BS, Pickel JM, Kilbey SM. Macromolecules 2009;42:3933–3941.
13. Sun B, Liu XH, Buck ME, Lynn DM. Chem Commun 2010;46:2016–2018.
14. De Geest BG, Skirtach AG, Mamedov AA, Antipov AA, Kotov NA, De Smedt SC, Sukhorukov GB. Small 2007;3:804–808. [PubMed: 17385759]
15. (a) Donath E, Sukhorukov GB, Caruso F, Davis SA, Möhwald H. Angew Chem Int Ed 1998;37:2201–2205. (b) Caruso F, Trau D, Möhwald H, Renneberg R. Langmuir 2000;16:1485–1488. (c) Antipov, Aa; Sukhorukov, GB.; Donath, E.; Möhwald, H. J Phys Chem B 2001;105:2281–2284. (d) Schüler C, Caruso F. Biomacromolecules 2001;2:921–926. [PubMed: 11710050] (e) Balabushevitch NG, Sukhorukov GB, Moroz Na, Volodkin DV, Larionova NI, Donath E, Mohwald H. Biotechnol Bioeng 2001;76:207–213. [PubMed: 11668455] (f) Donath E, Moya S, Neu B, Sukhorukov GB, Georgieva R, Voigt A, Bäumler H, Kiesewetter H, Mohwald H. Chem Eur J 2002;8:5481–5485. (g) Antipov A, Shchukin D, Fedutik Y, Zanaveskina I, Klechkovskaya V, Sukhorukov G, Möhwald H. Macromol Rapid Commun 2003;24:274–277. (h) Johnston A, Cortez C, Angelatos A, Caruso F. Curr Opin Colloid Interface Sci 2006;11:203–209. (i) Kreft O, Georgieva R, Bäumler H, Steup M, Müller-Röber B, Sukhorukov GB, Möhwald H. Macromol Rapid Commun 2006;27:435–440.
16. Caruso F. Chem Eur J 2000;6:413–419.
17. Volodkin DV, Larionova NI, Sukhorukov GB. Biomacromolecules 2004;5:1962–1972. [PubMed: 15360312]
18. Petrov AI, Volodkin DV, Sukhorukov GB. Biotechnol Prog 2005;21:918–925. [PubMed: 15932274]
19. Caruso F, Caruso RA, Mohwald H. Chem Mater 1999;11:3309–3314.
20. Callister, WD. Material Science and Engineering: An Introduction. 6. John Wiley & Sons, Inc; New York, NY: 2003.

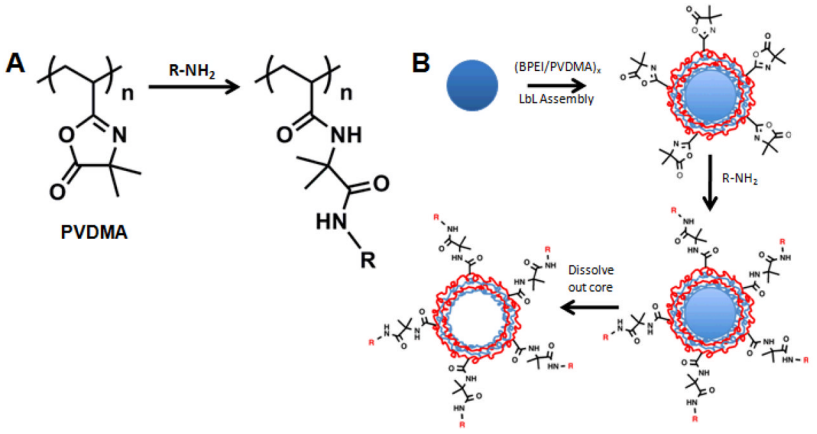

Figure 1.
(A) PVDMA undergoes rapid reactions with primary amines. (B) General scheme for fabricating hollow, reactive microcapsules.

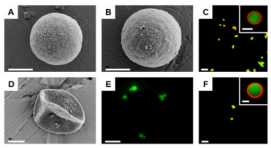

Figure 2.

Representative SEM images of (A) an uncoated $CaCO_3$ microparticle, (B) a $CaCO_3$ microparticle coated with a $(BPEI/PVDMA)_5$ film, and (D) a hollow microcapsule formed by dissolving a $(BPEI/PVDMA)_5$ coated $CaCO_3$ core using 0.2M EDTA. (C, F) Representative confocal microscopy images of $CaCO_3$ microparticles coated with $(BPEI/PVDMA)_5$ films reacted with TMR-cadaverine. The microparticle and microcapsule cores were labeled by encapsulation of FITC-dextran during fabrication (see text). (C) Low and high (inset) magnification images of coated $CaCO_3$ microparticles. (F) Low and high (inset) magnification images of hollow microcapsules formed by dissolving the $CaCO_3$ core. (E) Representative fluorescence microscopy image of hollow microcapsules prepared on $CaCO_3$ cores after removal of the cores and incubation in saturated aqueous NaCl for 22 hours. Scale bars = (A, B, D) 2 μm, (C, E, F) 20 μm, (insets) 3 μm.

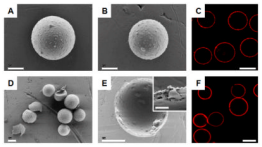

Figure 3.

Representative SEM images of (A) an uncoated glass microparticle, (B) a glass microparticle coated with a $(BPEI/PVDMA)_5$ film, and (D, E) hollow microcapsules formed by dissolving $(BPEI/PVDMA)_5$ coated glass cores using 10% HF. The inset of panel E shows a higher magnification view of the capsule wall. (C) Representative confocal microscopy images of glass microparticles coated with $(BPEI/PVDMA)_5$ films reacted with TMR-cadaverine. (F) Representative confocal microscopy image of hollow microcapsules formed by dissolving coated glass cores using 10% HF. $(BPEI/PVDMA)_5$ films were reacted with TMR-cadaverine prior to HF treatment. Scale bars = (A, B) 10 μm, (D, E) 20 μm, (inset) 2 μm, (C, F) 30 μm.

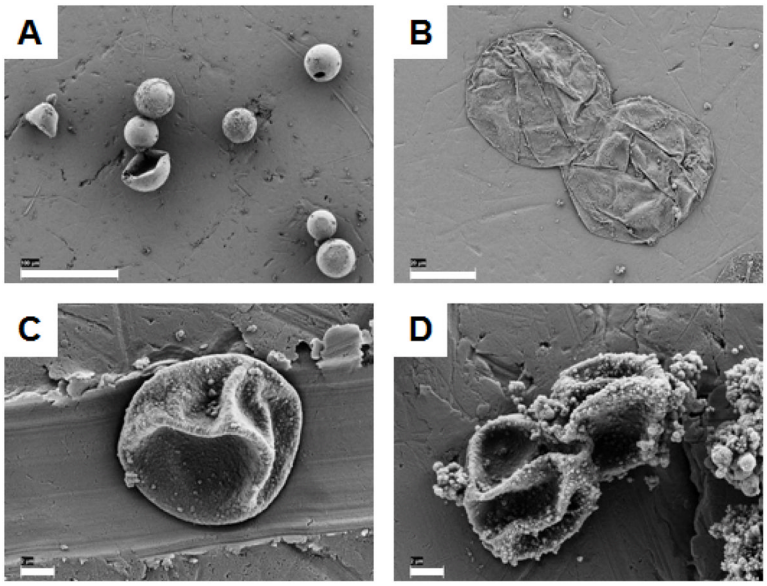

Figure 4.

(A, B) SEM image of hollow microcapsules formed by dissolving $(BPEI/PVDMA)_5$ coated glass beads in 10% HF. (A) Hollow microcapsules rinsed several times in water to remove residual HF. (B) Hollow microcapsules soaked in 0.2 M EDTA after removing the glass cores with HF. (C, D) SEM images of hollow microcapsules formed by dissolving $(BPEI/PVDMA)_5$ coated $CaCO_3$ microparticles in 0.2M EDTA. (C) Hollow microcapsules were treated with 10% HF for 24 hours. (D) ~3 mg of uncoated glass beads were added to the microcapsule suspension, and the suspension was treated with 10% HF for 24 hours. Scale bars = (A) 100 μm, (B) 20 μm, (C, D) 2 μm.

Table 1

Results of EDS Characterization of Glass Beads Coated with 5 Bilayers of BPEI/PVDMA*

Sample		Undissolved	HF Treated	HF Treated with H_2O Rinses	HF Treated with EDTA Rinses
Atom %	C	11.8 (± 0.4)	6.1 (± 2.4)	5.8 (± 2.7)	43.0 (± 2.8)
	N	9.6 (± 0.8)	5.8 (± 0.7)	5.6 (± 1.4)	11.1 (± 0.9)
	O	48.4 (± 3.9)	8.1 (± 1.5)	7.7 (± 1.7)	11.6 (± 2.1)
	F	0.2 (± 0.1)	47.6 (± 5.7)	46.8 (± 10.6)	17.9 (± 13.2)
	Na	3.4 (± 0.4)	0.4 (± 0.1)	0.2 (± 0.2)	4.0 (± 2.9)
	Mg	1.7 (± 0.2)	4.1 (± 0.2)	2.9 (± 1.0)	9.7 (± 2.7)
	Si	22.7 (± 3.3)	0.2 (± 0.1)	0.1 (± 0.0)	0.2 (± 0.2)
	Ca	2.2 (± 0.5)	27.7 (± 9.7)	30.8 (± 12.6)	2.4 (± 1.7)

*EDS data were collected at an accelerating voltage of 8 kV. Data are presented as mean atom % (± standard deviation) as determined by EDS analysis of at least 3 different microparticles or microcapsules per sample.