Tuning the Permeability of Polymer Hydrogel Capsules: An Investigation of Cross-Linking Density, Membrane Thickness, and Cross-Linkers

ARTICLE *in* LANGMUIR · MARCH 2011

Impact Factor: 4.46 · DOI: 10.1021/la104510e · Source: PubMed

4 AUTHORS, INCLUDING:

Alexander N Zelikin
Aarhus University
100 PUBLICATIONS **3,735** CITATIONS

SEE PROFILE

Langmuir
Article
pubs.acs.org/Langmuir

Tuning the Permeability of Polymer Hydrogel Capsules: An Investigation of Cross-Linking Density, Membrane Thickness, and Cross-Linkers

Siow-Feng Chong,[†] Ji Hyun Lee,[†] Alexander N. Zelikin,[†,‡] and Frank Caruso*[,†]

[†]*Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia, and* [‡]*Department of Chemistry and Interdisciplinary Nanoscience Centre (iNano), Aarhus University, Aarhus-C 8000, Denmark*

Received November 12, 2010. Revised Manuscript Received December 19, 2010

Nanoengineered poly(methacrylic acid) hydrogel capsules (PMA HCs) are promising candidate carriers for biomedical applications, especially in the areas of drug delivery, encapsulated catalysis, and cell mimicry. The assembly, stability, and degradation of these carriers, as well as their use for the encapsulation of therapeutics, have received considerable attention. However, tailoring the permeability properties of PMA HCs to various types of cargo remains largely unexplored. Herein, we investigate fundamental parameters that govern the structural integrity and the capability of PMA HCs to encapsulate macromolecular cargo. The thiol content of the constituent polymers and the number of deposited polymer layers are shown to be key factors in controlling cargo retention within the PMA HCs. We further introduce a new strategy to achieve disulfide cross-linking for PMA HCs via a thiol−disulfide exchange in order to obtain capsules with superior cargo retention characteristics. Finally, we provide evidence for the semipermeable nature of PMA HCs based on the charge of the solutes and demonstrate that rational design of these systems can yield capsules with specific cargo retention properties. This work contributes toward the development of multilayered polymer capsules and PMA HCs and associated applications in biomedicine.

Introduction

Nanoengineered capsules assembled via layer-by-layer (LbL) deposition of polymers onto sacrificial colloidal substrates[1,2] have attracted interest for their use as carrier vessels in areas such as therapeutic delivery,[3−6] microreactions,[7,8] and artificial cells.[9] For drug delivery applications, capsules ideally isolate the drug from the body, preventing early therapeutic release and reducing side effects. In addition, they provide opportunities for controlled drug release[10,11] and surface modification for targeted drug delivery.[12] For catalysis, capsules allow isolated chemical reactions to be carried out from within their confines.[13] When engineered to contain multiple subcompartments,[14] these carries have the potential to host multiple catalytic reactions within a single capsule, aiding progress in the creation of artificial cells.[15]

Among the successful candidates for drug carriers, polymer capsules comprised of disulfide-cross-linked poly(methacrylic acid) (PMA_{SH})[16,17] exhibit high colloidal stability[18] and controlled degradability[19]—two crucial parameters toward the successful use of a carrier system in biomedical applications. Poly(methacrylic acid) hydrogel capsules (PMA HCs) have already shown significant promise as drug delivery vehicles[8,18,20−25] and microreactors,[8,9,15] largely due to the successful encapsulation of diverse biomolecules such as DNA,[25] oligoneucleotides,[21−23] proteins,[24] and anticancer drugs.[20] For microreactor and artificial cell development, LbL-derived HCs afford the incorporation of multiple subcompartments composed of liposomes[9,15] and smaller HCs.[14] In these studies, the properties of PMA HCs, such as stability, cargo loading, and degradability, have been investigated. In contrast, control over the permeability of these HCs to diverse cargoes remains largely unexplored and therefore warrants further investigation. In particular, understanding and controlling the permeability of

*Corresponding author. E-mail: fcaruso@unimelb.edu.au.

(1) Caruso, F.; Caruso, R. A.; Möhwald, H. *Science* **1998**, *282*, 1111.
(2) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S. A.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2202.
(3) Such, G. K.; Johnston, A. P. R.; Caruso, F. *Chem. Soc. Rev.* **2011**, *40*, 19.
(4) Becker, A. L.; Johnston, A. P. R.; Caruso, F. *Small* **2010**, *6*, 1836.
(5) Del Merceto, L. L.; Rivera-Gil, P.; Abbasi, A. Z.; Ochs, M.; Ganas, C.; Zins, I.; Sönnichsen, C.; Parak, W. J. *Nanoscale* **2010**, *2*, 458.
(6) De Cock, L. J.; De Koker, S.; De Geest, B. G.; Grooten, J.; Vervaet, C.; Remon, J. P.; Sukhorukov, G. B.; Antipina, M. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 6954.
(7) Wang, B.; Zhao, Q.; Wang, F.; Gao, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1560.
(8) Price, A. D.; Zelikin, A. N.; Wang, Y.; Caruso, F. *Angew. Chem., Int. Ed.* **2009**, *48*, 329.
(9) Städler, B.; Chandrawati, R.; Price, A. D.; Chong, S.-F.; Breheney, K.; Postma, A.; Connal, L. A.; Zelikin, A. N.; Caruso, F. *Angew. Chem., Int. Ed.* **2009**, *48*, 4359.
(10) Angelatos, A. S.; Radt, B.; Caruso, F. *J. Phys. Chem. B* **2005**, *109*, 3071.
(11) Wang, Y.; Yan, Y.; Cui, J.; Hosta-Rigau, L.; Heath, J. K.; Nice, E. C.; Caruso, F. *Adv. Mater.* **2010**, *22*, 4293.
(12) (a) Cortez, C.; Tomaskovic-Crook, E.; Johnston, A. P. R.; Radt, B.; Cody, S. H.; Scott, A. M.; Nice, E. C.; Heath, J. K.; Caruso, F. *Adv. Mater.* **2006**, *18*, 1998. (b) Kamphuis, M. M. J.; Johnston, A. P. R.; Such, G. K.; Dam, H. H.; Evans, R. A.; Scott, A. M.; Nice, E. C.; Heath, J. K.; Caruso, F. *J. Am. Chem. Soc.* **2010**, *132*, 15881. (c) Cortez, C.; Tomaskovic-Cook, E.; Johnston, A. P. R.; Scott, A. M.; Nice, E. C.; Heath, J. K.; Caruso, F. *ACS Nano* **2007**, *1*, 93.
(13) Price, A. D.; Zelikin, A. N.; Wark, K. L.; Caruso, F. *Adv. Mater.* **2010**, *22*, 720.
(14) Kulygin, O.; Price, A. D.; Chong, S.-F.; Städler, B.; Zelikin, A. N.; Caruso, F. *Small* **2010**, *10*, 1558.
(15) Chandrawati, R.; Städler, B.; Postma, A.; Connal, L. A.; Chong, S.-F.; Zelikin, A. N.; Caruso, F. *Biomaterials* **2009**, *30*, 5988.
(16) Zelikin, A. N.; Price, A. D.; Städler, B. *Small* **2010**, *6*, 2201.
(17) Zelikin, A. N.; Quinn, J. F.; Caruso, F. *Biomacromolecules* **2006**, *7*, 27.
(18) Zelikin, A. N.; Li, Q.; Caruso, F. *Chem. Mater.* **2008**, *20*, 2655.
(19) Becker., A. L.; Zelikin, A. N.; Johnston, A. P. R.; Caruso, F. *Langmuir* **2009**, *25*, 14079.
(20) Sivakumar, S.; Bansal, V.; Cortez, C.; Chong, S.-F.; Zelikin, A. N.; Caruso, F. *Adv. Mater.* **2009**, *21*, 1820.
(21) Chong, S.-F.; Sexton, A.; De Rose, R.; Kent, S. J.; Zelikin, A. N.; Caruso, F. *Biomaterials* **2009**, *30*, 5178.
(22) Sexton, A.; Whitney, P. G.; Chong, S.-F.; Zelikin, A. N.; Johnston, A. P. R.; De Rose, R.; Brooks, A. G.; Caruso, F.; Kent, S. J. *ACS Nano* **2009**, *3*, 3391.
(23) De Rose, R.; Zelikin, A. N.; Johnston, A. P. R.; Sexton, A.; Chong, S.-F.; Cortez, C.; Mulholland, W.; Caruso, F.; Kent, S. J. *Adv. Mater.* **2008**, *20*, 4698.
(24) Zelikin, A. N.; Li, Q.; Caruso, F. *Angew. Chem.* **2006**, *118*, 7907.
(25) Zelikin, A. N.; Becker, A. L.; Johnston, A. P. R.; Wark, K. L.; Turatti, F.; Caruso, F. *ACS Nano* **2007**, *1*, 63.

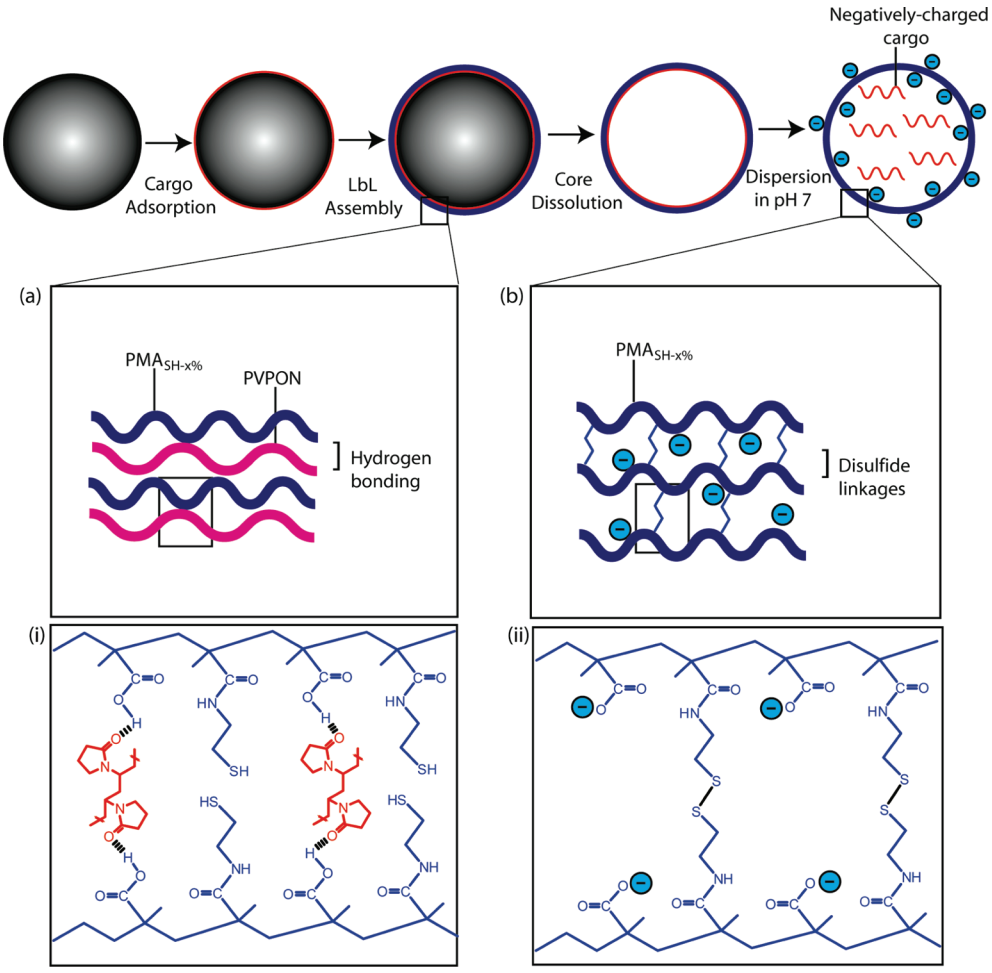

Figure 1. Assembly of PMA HCs. (a) Schematic illustration of a PMA_{SH}/PVPON multilayer film and (b) stabilization of PMA_{SH} layers through disulfide linkages. Chemical interactions between (i) PMA_{SH} and PVPON and (ii) PMA_{SH} layers.

PMA HCs is important as it ensures retention of cargo molecules during cellular delivery. For applications in microencapsulated catalysis, controlled permeability affords retention of catalytic machinery, while allowing diffusion of reagents and reaction products, which provides a platform for the engineering of artificial cells.

In moving toward this goal, we examine the permeability of PMA HCs and engineer carrier vessels that are either permeable to macromolecular cargo or impermeable (sealed and retain encapsulated material without leakage). Furthermore, we present PMA HCs that are semipermeable to macromolecular cargo based on the charge of the encapsulated materials (negative or noncharged), a system with high potential in the creation of synthetic microreactors. To achieve this, we take advantage of the LbL technique to control the thickness of the capsule membrane via the number of deposited polymer layers. An alternate method is to vary the degree of thiolation of PMA, which translates into different cross-linking densities of the PMA HCs. Finally, we introduce a novel technique to effect conversion of thiol groups within the structure of the multilayered polymer film into bridging disulfide linkages, which affords PMA HCs with superior cargo retention. Taken together, the presented data significantly contribute toward the development of PMA HCs and other multilayered polymer capsules, with the aim to applying these systems in diverse biomedical applications.

Experimental Section

Materials. Unless stated otherwise, all chemicals and materials were purchased from Sigma-Aldrich and used as received without purification. 1 and 3 μm diameter SiO_2 particles were purchased from MicroParticles GmbH (Berlin, Germany). Poly(methacrylic acid, sodium salt) (PMA), M_w = 15 kDa, was purchased from Polysciences. Alexa Fluor 488 cadaverine, sodium salt (AF488-C), and phosphate-buffered saline (PBS) were obtained from Invitrogen. Ethylenediaminetetraacetic acid (EDTA), 2-(*N*-morpholine)ethanesulfonic acid (MES), 3-morpholinopropane-1-sulfonic acid (MOPS), sodium acetate (NaOAc), and 2-amino-2-hydroxymethylpropane-1,3-diol (Tris) were purchased from Merck. Pyridine dithioethylamine hydrochloride (PDA) was purchased from Shanghai Speed-Chemical Co. Ltd., China. AF488-labeled poly(vinylpyrrolidone) (AF488-PVPON), M_w = 10 kDa, was synthesized according to previously published protocols.[18] High-purity water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore Synergy system (Millipore Pty Ltd., AUS).

Preparation of PMA_{SH}. PMA samples with varying degrees of thiol modification (mol %) were synthesized through pendant functionalization of PMA with PDA. In particular, 67 mg of PMA was diluted into 1.9 mL of phosphate buffer (10 mM, pH 7.2). The resulting solution was incubated with *N*-(3-(dimethylamino)-propyl)-*N*′-ethylcarbodiimide (EDC), 3 times in molar excess of the target modification, for 15 min. Subsequently, PDA (target modifications of 5, 9, 12, and 17 mol %) was added to the mixture, and the reaction was allowed to proceed overnight. The reaction mixture was purified via dialysis for 2 days against water and freeze-dried to obtain a white powder of PMA_{PD}. The thiol content of the resulting polymer was characterized by measuring the absorbance of the released chromophore, 2-pyridinethione (λ_{max} = 343 nm), and then quantified from a calibration curve of PDA.

Fluorescent labeling of PMA was carried out by incubating 45 mg of PMA (10 g L^{-1}) in phosphate buffer (0.1 M, pH 8) with EDC for 15 min. The reaction solution was then added to 100 μL of AF488-C (1 g L^{-1} in water), and the reaction was allowed to proceed overnight. The resulting polymer was collected in Tris−EDTA buffer (10 mM, pH 7.5) through purification via size exclusion chromatography (SEC) using a NAP-5 desalting column.

The polymer (PMA_{SH}) was dissolved at a concentration of 50 g L^{-1} with 0.5 M of DTT solution in MOPS buffer (20 mM, pH 8) for at least 15 min at 37 °C in order to expose thiol groups prior to LbL assembly.

Adsorption of Fluorescently Labeled Cargo and Assembly of Multilayer Films. A stock solution of PVPON (M_w = 10 kDa, 100 g L^{-1}) was prepared in Milli-Q water. In a typical experiment, a suspension of 1 and 3 μm diameter SiO_2 particles (5 wt % suspension) were washed twice and dispersed in a 2 g L^{-1} solution of NaOAc buffer (20 mM, pH 4) to a concentration of 10 wt %. An equal volume of PVPON (or AF488-PVPON) solution (2 g L^{-1}) in NaOAc buffer was then added and incubated for 10 min with constant shaking to facilitate polymer adsorption. The PVPON-coated particles were then washed three times and coated with 1 g L^{-1} of AF488-PMA (or $PMA_{SH\text{-}x\%}$ for AF488-PVPON adsorbed particles). The suspension was incubated for 15 min, washed three times, and redispersed in pH 4 buffer. The outlined procedure describes the assembly of a single polymer bilayer. The adsorption of subsequent interacting polymers ($PMA_{SH\text{-}x\%}$/PVPON) was repeated until the desired number of layers was achieved.

Multilayer Cross-Linking. Different cross-linking strategies were used to stabilize the multilayered polymer film. Disulfide-stabilized hydrogel capsules were obtained by exposing particles to a 2.75 mM solution of chloramine T (CaT) in MES buffer solution (20 mM, pH 6) for 1 min. Cross-linking through a pyridyl disulfide reaction used an excess volume of 2,2′-dithiodipyrridine (DTDP) in NaOAc buffer at varying concentrations (0.03−2.7 g L^{-1}), and the reaction was left to proceed overnight with constant shaking.

After completion of multilayer cross-linking, the particles were washed and redispersed into pH 4 buffer. The silica template particles were dissolved by treatment with 5 M of aqueous HF, and the obtained capsules were washed with at least four washing cycles with NaOAc buffer. (*Caution! Hydrofluoric acid is highly toxic. Extreme care should be taken when handling HF solution, and only small quantities should be prepared.*)

Characterization Methods. Absorbance measurements were performed using a UV−vis spectrophotometer (NanoDrop). Flow cytometry analysis was performed on a CyFlow Space (Partec GmbH) flow cytometer using an excitation wavelength of 488 nm. In each case at least 15 000 events were analyzed. Particles were imaged on an Olympus IX71 digital wide-field fluorescence microscope with a fluorescent filter cube.

Results and Discussion

The capsule wall thickness, which is controlled by the number of deposited polymer bilayers,[26,27] is a primary parameter for control over permeability of multilayered polymer capsules. For PMA HCs, another factor relates to the cross-linking nature of the hydrogel capsule wall, where cross-linking density provides a size exclusion mechanism based on the hydrogel mesh size. In order to investigate this effect, a straightforward approach is to use PMA with varying degrees of thiolation; however, this strategy brings about diverse effects with respect to the stability of the HCs. Thus, stabilization of PMA HCs relies on fine-tuning the polymers with thiol groups, deposition of these polymers to form a multilayered film, and conversion of the thiols into bridging disulfide linkages.[17] Upon the introduction of thiol moieties through reactions with the carboxylic groups on the polymer chain, PMA exhibits a decreased hydrophilicity and possibly an increased degree of polymer chain coiling. In agreement with this, we have previously observed a significant increase in the mass of a single deposited polymer layer with increasing PMA thiol content.[19] Furthermore, an increase in polymer thiolation is expected to afford a denser cross-linking of the hydrogel, i.e., a hydrogel membrane with a smaller mesh size. Both intra- and interlayer cross-linking of polymer chains can be impeded by the adsorption of polymers in their (coiled) compact conformation. Therefore, investigation of the role of polymer thiolation on hydrogel permeability merits consideration. This effect may be used in combination with the number of deposited polymer layers to engineer capsules with desired permeability properties.

To study the correlation between the degree of PMA thiol modification and HC cross-linking density, PMA samples with varied thiol content were prepared via a polymer-analogous reaction, i.e., pendant functionalization using carbodiimide-mediated coupling. Preparation of PMA_{SH} was achieved via a procedure adopted from our previous work on the use of pyridine dithioethylamine hydrochloride (PDA) to obtain PMA samples with 5, 9, 12, and 17 mol % thiol modification ($PMA_{SH\text{-}x\%}$, where x corresponds to mol % thiol).[19] As an increasing degree of thiol substitution enhances polymer hydrophobicity, and consequently decreases solubility, PMA samples were limited to an activated thiol content of 20 mol %.

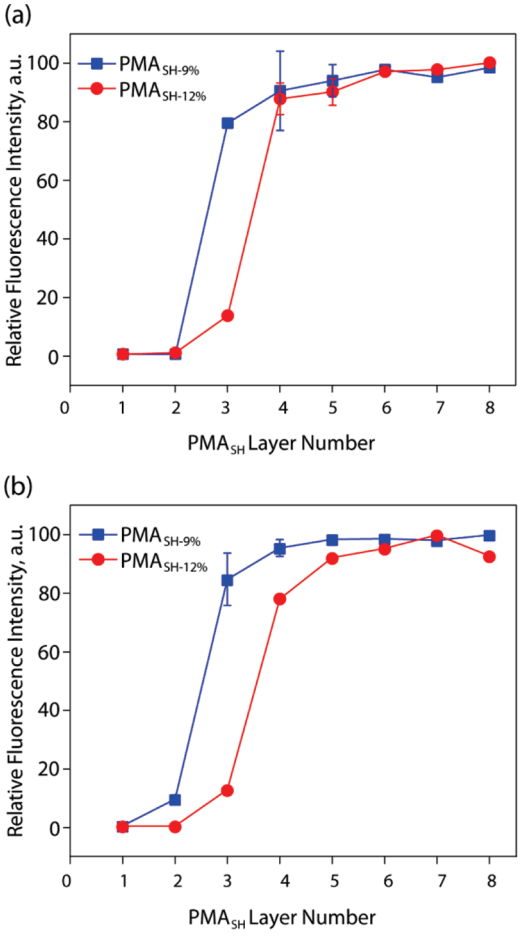

Figure 2. Encapsulation efficiency of 15 kDa PMA within (a) 3 μm and (b) 1 μm diameter disulfide-cross-linked HCs composed of $PMA_{SH\text{-}9\%}$ (squares) and $PMA_{SH\text{-}12\%}$ (circles) at pH 7.4. Retention of PMA is achieved by electrostatic repulsion and increased capsule wall thickness, the latter determined by layer number.

(26) Antipov, A. A.; Sukhorukov, G. B.; Leporatti, S.; Radtchenko, I. L.; Donath, E.; Möhwald, H. *Colloids Surf., A* **2002**, *198*, 535.
(27) Johnston, A. P. R.; Caruso, F. *J. Am. Chem. Soc.* **2005**, *127*, 10014.

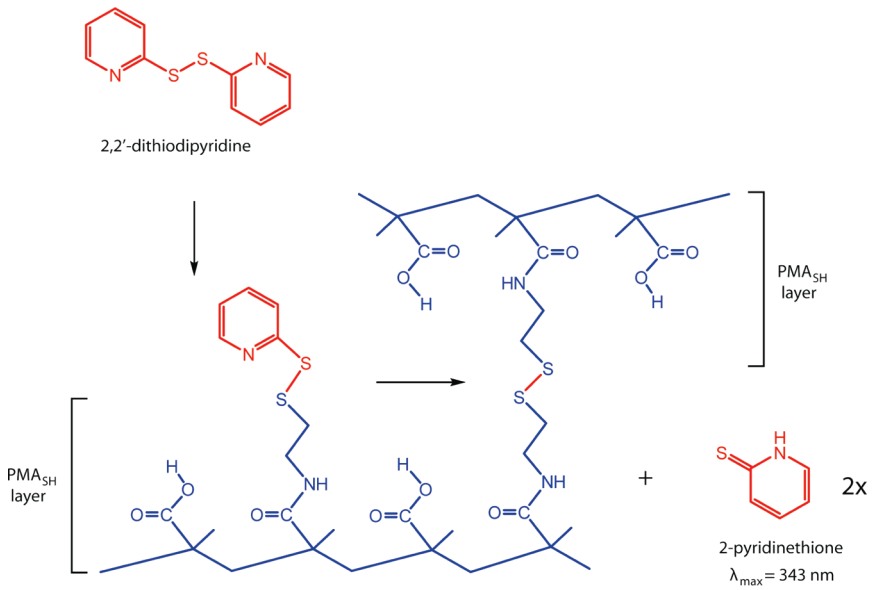

Figure 3. Schematic of chemical interactions between PMA_{SH} and the cross-linker 2,2′-dithiodipyridine (DTDP).

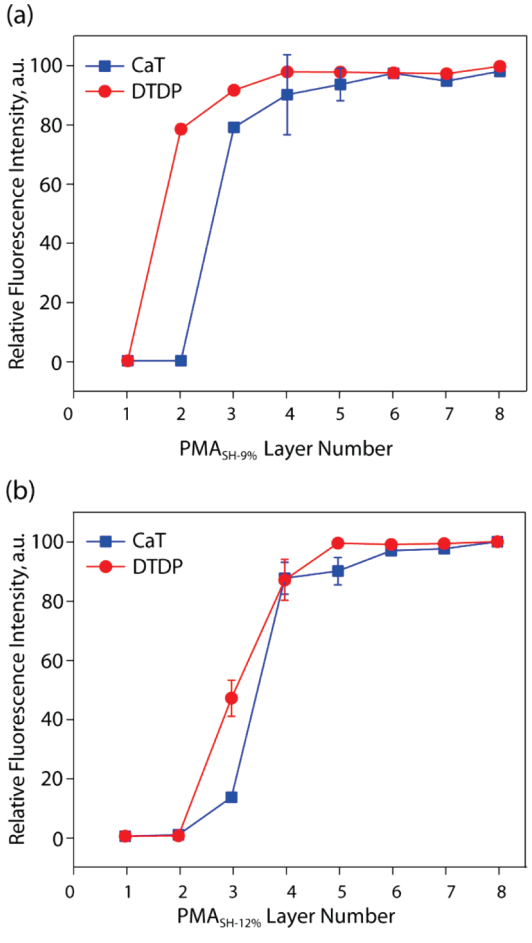

Figure 4. Encapsulation efficiency of 15 kDa PMA within 3 μm diameter HCs composed for (a) $PMA_{SH-9\%}$ and (b) $PMA_{SH-12\%}$ polymers, cross-linked with CaT (squares) or DTDP (circles).

Successful encapsulation of therapeutics within polymer HCs relies on a firm understanding of capsule permeability. In our previous reports, we demonstrated this through investigations of PMA HCs with a negatively charged capsule membrane, encapsulating like-charged cargo (i.e., DNA[25] and PMA[21−23]) (Figure 1b).

Table 1. Size of PMA HCs Stabilized by the Cross-Linkers DTDP and CaT[a]

number of $PMA_{SH-9\%}$ layers	size of PMA HCs (μm)		
	DTDP		
	0.03 g L^{-1}	1.3 g L^{-1}	CaT
2	3.83 ± 0.31	4.23 ± 0.23	N/A
3	4.27 ± 0.31	3.99 ± 0.29	4.21 ± 0.36
4	4.60 ± 0.28	3.88 ± 0.23	4.20 ± 0.33
5		3.93 ± 0.27	4.19 ± 0.25
6		3.92 ± 0.23	4.21 ± 0.25
7		3.92 ± 0.61	4.13 ± 0.35
8		3.80 ± 0.39	3.85 ± 0.35

[a] Capsule sizes represent the average diameters determined from 15 PMA HCs.

In this instance encapsulation is effective and reliable when a sufficient degree of charge repulsion and hydrogel cross-linking density are present. In other words, both size exclusion and charge repulsion are identified as two important factors contributing to the permeability characteristics of PMA HCs. In the current study, we monitor encapsulation, retention, and release of model cargo to ascertain the permeability of PMA HCs. We used fluorescently labeled cargo and flow cytometry to quantify the fluorescence of individual capsules and fluorescence microscopy to provide visual proof of encapsulation and cargo retention. Nonfunctionalized, pristine PMA (15 kDa, as the model cargo) was labeled with a fluorophore (AF488) and deposited onto silica particles (1 and 3 μm diameter) which were precoated with a layer of PVPON. The particles obtained were further used to deposit layers of $PMA_{SH-x\%}$ and PVPON (Figure 1a). After each deposited $PMA_{SH-x\%}$/PVPON bilayer, an aliquot of particles was taken, and the multilayer was oxidized using chloramine T (CaT). To study cargo encapsulation for all experiments described below, template particles were removed using HF, and the resulting capsules were washed with pH 7.4 PBS. Under these conditions, hydrogen bonding between the two polymers, $PMA_{SH-x\%}$ and PVPON, becomes inefficient, and PVPON is thus expelled from the multilayer film, yielding single-component PMA HCs (Figure 1b). Using this strategy, we have previously demonstrated that deposition of as few as two bilayers of $PMA_{SH-12\%}$/PVPON affords capsules with structural integrity;[19,21] however, the full retention

of encapsulated 15 kDa PMA cargo necessitates HCs composed of at least five $PMA_{SH\text{-}12\%}$ layers.[21] Herein, we used a similar approach to monitor the permeability of the $PMA_{SH\%}$ capsules assembled with varying degrees of thiolation.

The first observation is that for PMA HCs assembled from 5 mol % thiolation no fluorescence was detected within the capsules, regardless of the number of deposited layers (up to eight $PMA_{SH\text{-}5\%}$ layers, data not shown). This is in agreement with our previous study, where a 5 mol % thiolated sample of PMA did not lead to the formation of robust PMA HCs.[19] This observation suggests that for these PMA HCs the multilayer thickness alone is unable to provide an efficient barrier for the encapsulation of 15 kDa PMA cargo. Similar results were obtained for capsules using $PMA_{SH\text{-}17\%}$; that is, these HCs did not successfully encapsulate and retain the PMA cargo (data not shown). Previously, we showed that these capsules possess a thick membrane wall, possibly due to the compact conformation of the adsorbing polymer chains.[19] Nonetheless, the assembly of these polymer layers leads to inefficient intra- and interlayer cross-linking. As above, this observation also implies that the multilayer thickness alone is not sufficient to provide an impermeable barrier allowing complete cargo retention. Even with a thick hydrogel membrane, diffusion to the external environment can be unhindered, most likely due to the large mesh size of the hydrogel. For PMA HCs assembled from $PMA_{SH\text{-}9\%}$ and $PMA_{SH\text{-}12\%}$ (Figure 2), encapsulation efficiencies follow the trend reported previously,[21] as capsules composed of four to five PMA_{SH} layers quantitatively retain the encapsulated 15 kDa PMA cargo. Interestingly, $PMA_{SH\text{-}9\%}$ appears to be better suited for encapsulation applications. With four adsorbed polymer bilayers, the capsules assembled on both the 1 and 3 μm diameter templates exhibit sufficient retention capability (over 90% of encapsulation efficiency); that is, these capsules retain their cargo over 24 h of incubation in pH 7. On the other hand, HCs composed from $PMA_{SH\text{-}12\%}$ require an additional PMA_{SH} layer to retain their contents to the same degree. The improvement of encapsulation efficiency with 9 mol % of thiolation is probably due to the combined effects of enhanced cross-linking and charge density of the hydrogel.

The results presented above suggest that cross-linking efficiency can play a pivotal role in the permeability of PMA HCs. We have previously reported several novel cross-linking strategies to obtain PMA HCs, including the use of thiol-activated polymers,[28] bis-maleimide homobifunctional cross-linkers,[14] and the best studied approach to date, presented above, using an oxidizing agent, CaT. The latter affords PMA HCs stabilized with disulfide linkages, which are of interest in biomedical applications.[29] Also in this work, we aimed to optimize disulfide cross-linking in PMA HCs through the introduction of a novel cross-linking strategy, thiol−disulfide exchange using 2,2′-dithiodipyridine (DTDP). In developing this strategy, we built upon our prior experiments using polymeric pyridyl disulfide functionalized cross-linkers[28] and produced pyridyl disulfide-activated thiols in situ within the structure of the multilayers using DTDP (Figure 3). Upon the addition of DTDP, the cross-linker infiltrates into the multilayers and reacts with a thiol group, which is intrinsically active toward thiol−disulfide exchange. Subsequent reaction with a neighboring thiol group affords polymer cross-linking, i.e., disulfide stabilization of the PMA HCs. To accomplish this, the rate of the second reaction has to be high enough to achieve efficient disulfide cross-linking before the neighboring thiol group reacts with another DTDP molecule. This can be overcome by a high

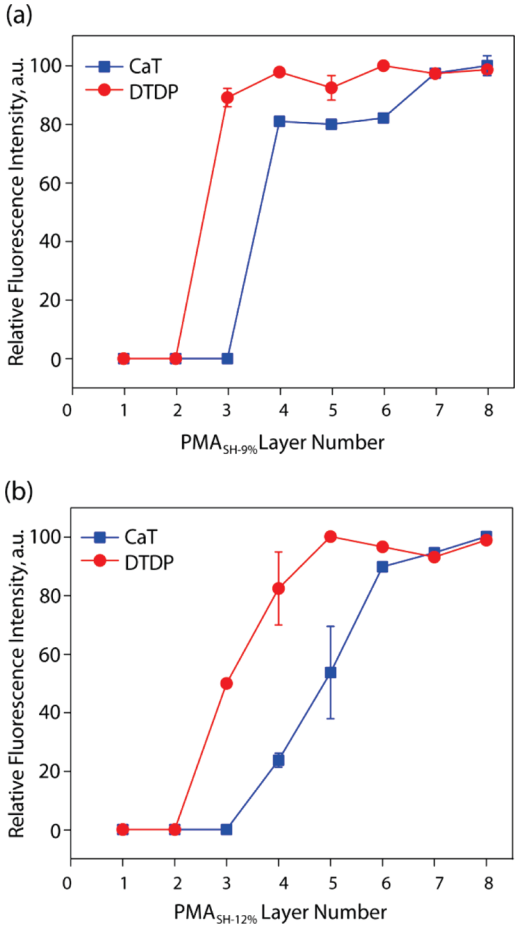

Figure 5. Encapsulation efficiency of 10 kDa PVPON within (a) 3 μm and (b) 1 μm diameter HCs composed of $PMA_{SH\text{-}9\%}$, cross-linked with CaT (squares) and DTDP (circles). Retention of cargo is achieved by improved cross-linking density and increased capsule wall thickness, the latter determined by layer number.

local concentration of thiol groups within the structure of PMA HCs or with a low concentration of DTDP (discussed below). Unlike oxidation, thiol−disulfide exchange can be carried out under a wide range of conditions. It is a thiol-specific reaction, largely insensitive to the presence of other reactive groups, and does not affect fragile cargo, e.g. encapsulated enzymes.[28]

In this work, we compare DTDP with the oxidizing agent CaT, specifically with respect to its ability to afford PMA HCs with cargo retention capabilities with the minimum number of PMA_{SH} layers. To this end, the cargo-immobilized and multilayer-assembled particles were individually incubated with CaT (pH 6 buffer for 1 min) and DTDP (pH 4 buffer, overnight). With 1.3 g L^{-1} of DTDP, the resulting HCs fabricated using both $PMA_{SH\text{-}9\%}$ and $PMA_{SH\text{-}12\%}$ demonstrated a higher efficiency of cargo retention when compared to those prepared using CaT (Figure 4). This indicates the potential of DTDP as a more effective cross-linker at low numbers of PMA_{SH} layers. To increase the efficiency of multilayer cross-linking, we examined 3 μm diameter capsules formed from two layers of $PMA_{SH\text{-}9\%}$ with varying cross-linker concentrations. As the concentration of DTDP decreases from 1.3 to 0.03 g L^{-1}, the efficiency of cargo retention increases by 10% (Supporting Information, Figure S1). One possible explanation is that when excess cross-linker was introduced into the thiolated membrane, most of the thiol groups interact with the cross-linkers instantaneously, resulting in a low amount of free thiol left for the formation of bridging disulfides. We note that this

(28) Chong, S.-F.; Chandrawati, R.; Städler, B.; Park, J.; Cho, J.; Wang, Y.; Jia, Z.; Bulmus, V.; Davis, T. P.; Zelikin, A. N.; Caruso, F. *Small* **2009**, *5*, 2601.
(29) Saito, G.; Swanson, J. A.; Lee, K.-D. *Adv. Drug Delivery Rev.* **2003**, *55*, 199.

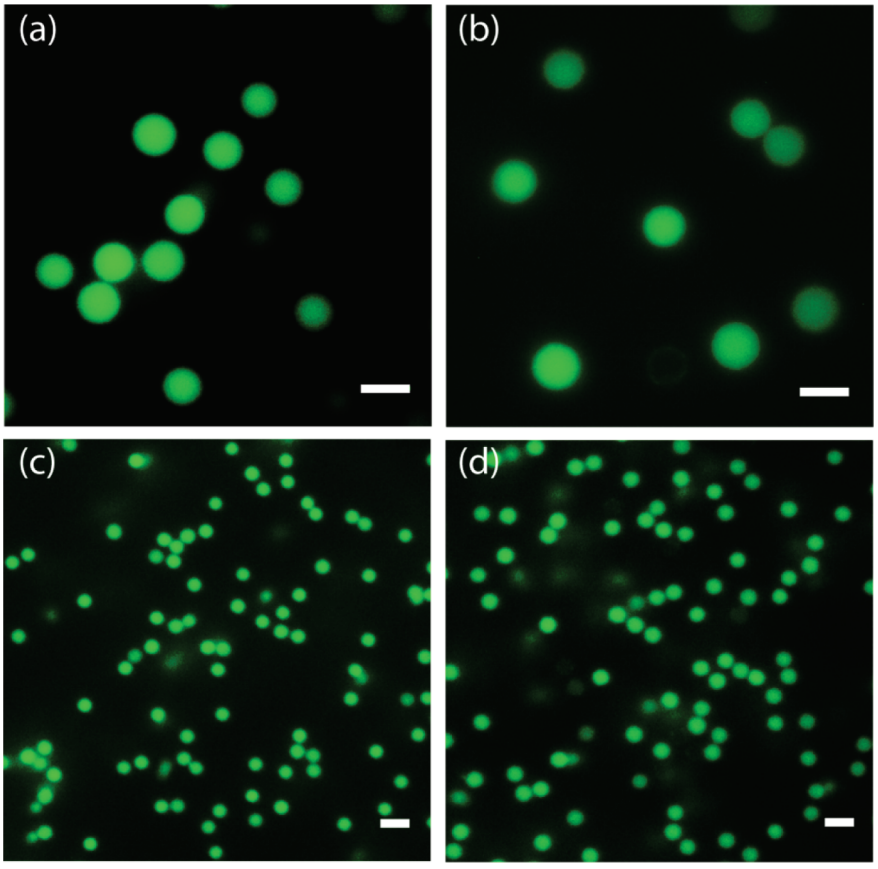

Figure 6. Fluorescence microscopy images of PMA HCs encapsulated with fluorescently labeled PVPON: (a) 3 μm and (c) 1 μm diameter HCs composed of five layers of $PMA_{SH\text{-}9\%}$, cross-linked with DTDP; (b) 3 μm and (d) 1 μm diameter HCs composed of eight layers of $PMA_{SH\text{-}9\%}$, cross-linked with CaT. Scale bars are 5 μm (top row) and 3 μm (bottom row).

10% difference allows PMA HCs to fully encapsulate the cargo of interest with no observable leakage over an extended period of incubation at pH 7. That is, cargo retention capabilities of lower than 90% will result in constant diffusion of cargo until the differential osmotic pressure driving force, inside and outside of the carrier, reaches equilibrium.

The cross-linking density of the hydrogel membrane can be qualitatively assessed by the size of the capsules at pH 7. At this pH, hydrogen bonding between the two polymers is disrupted, leaving only disulfide bonds to maintain capsule stability. As a result, the degree of swelling of a capsule reflects the cross-linking density of the capsule membrane.[30] With a lower concentration of DTDP (0.03 g L^{-1}), the capsules obtained with two $PMA_{SH\text{-}9\%}$ layers exhibited diminished swelling, as shown in Table 1, which is consistent with the cargo encapsulation efficiency characteristics discussed above. This suggests a higher cross-linking density resulted from the low concentration of DTDP used. Nonetheless, with higher thiol content (e.g., more than two layers of $PMA_{SH\text{-}9\%}$ or polymer chains with higher thiol modification), 0.03 g L^{-1} of DTDP may not be sufficient to create highly dense HCs. This is shown by the increasing size of capsules from two to four $PMA_{SH\text{-}9\%}$ layers. The CaT-treated capsules formed with $PMA_{SH\text{-}9\%}$ exhibited a slightly greater degree of swelling (on average) in comparison to DTDP-cross-linked capsules. These results substantiate that DTDP is an efficient cross-linker for the stabilization of PMA HCs.

As discussed above, we have outlined three controllable parameters (thiol content, membrane thickness, and cross-linkers) for the incorporation of negatively charged molecules into PMA HCs. To further investigate the capacity of PMA HCs for cargo retention, we employed poly(vinylpyrrolidone) (PVPON) with a molecular weight of 10 kDa as a second model cargo. PVPON is an FDA (Food and Drug Administration) approved polymer that is widely used in the pharmaceutical and cosmetic fields. Its biocompatibility allows potential applications as a drug carrier,[31,32] plasma expander,[33] and binder,[34] among others. Previously, we noted that increasing the pH gives way to diffusion of PVPON from HCs composed of $PMA_{SH\text{-}12\%}$.[18] Hence the encapsulation of PVPON, a promising polymer carrier, remains a challenge to date. Herein, we combined a polymer with higher retention capabilities, $PMA_{SH\text{-}9\%}$, and a novel cross-linking strategy, with DTDP to create HCs with greatly diminished permeability. Indeed, $PMA_{SH\text{-}9\%}$ alone afforded encapsulation of PVPON (in contrast to $PMA_{SH\text{-}12\%}$);[18] nonetheless, with the developed protocol PMA HCs were shown to have consistently lower permeability, as can be seen in Figure 5. We note that in this case some of the PVPON adsorbed for the assembly of the PMA HCs may be retained within the layers due to the decreased permeability of the capsules. For both 1 and 3 μm diameter HCs, the number of polymer layers required for quantitative retention of cargo was lower compared with HCs obtained using CaT. Fluorescence microscopy was used to visualize the capsules with encapsulated

(30) Kozlovskaya, V.; Kharlampieva, E.; Mansfield, M. L.; Sukhishvili, S. A. *Chem. Mater.* **2006**, *18*, 328.

(31) Monfardini, C.; Veronese, F. M. *Bioconjugate Chem.* **1998**, *9*, 418.

(32) Kaneda, Y.; Tsutsumi, Y.; Yoshioka, Y.; Kamada, H.; Yamamoto, Y.; Kodaira, H.; Tsunoda, S.; Okamoto, T.; Mukai, Y.; Shibata, H.; Nakagawa, S.; Mayumi, T. *Biomaterials* **2004**, *25*, 3259.

(33) Ravin, H. A.; Seligman, A. M.; Fine, J. N. *Engl. J. Med.* **1952**, *247*, 921.

(34) Ganesan, S.; Felo, J.; Saldana, M.; Kalasinsky, V. F.; Lewin-Smith, M. R.; Tomashefski, J. F. J., Jr. *Mod. Pathol.* **2003**, *16*, 286.

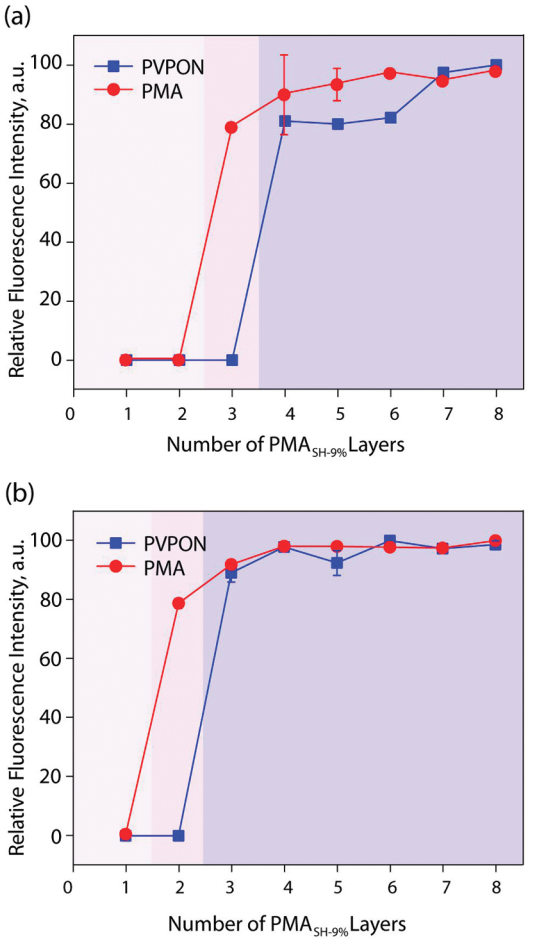

Figure 7. Encapsulation efficiency of 15 kDa PMA (circles) and 10 kDa PVPON (squares) within 3 μm diameter HCs composed of PMA$_{SH-9\%}$, cross-linked with (a) CaT and (b) DTDP. Permeability characteristics of the PMA HCs was divided into three regions: permeable, semipermeable, and impermeable.

cargo after 22 h of incubation in PBS (Figure 6), and the images obtained fully support the data in Figure 5, i.e., retention of fluorescent cargo within the HCs.

Comparing the retention of both cargos, PMA and PVPON, within HCs composed of PMA$_{SH-9\%}$ and cross-linked with DTDP, Figure 7 highlights the semipermeable nature of the HCs. For both candidate cargo molecules, judicious choice of the deposited polymer layers affords capsules with a permeable (no cargo retention, low number of deposited layers) or sealed (quantitative cargo retention, high number of layers) hydrogel membrane. Significantly, rational design of the HC membrane may allow capsules to retain one of the solutes, PMA, while being permeable to another, PVPON, through control over the number of deposited polymer layers. Such capsules may prove to be of particular importance in the development of encapsulated microreactors with controlled and uncoupled permeability characteristics for diverse cargo.

Conclusions

We studied several parameters that govern the stability and permeability of PMA HCs, namely, the degree of thiolation of the constituent polymers, the number of deposited polymer layers, and the nature of the encapsulated cargo. To improve cargo retention capabilities, we introduced a new cross-linking strategy based on thiol−disulfide exchange. With the optimized cross-linking strategy, we successfully demonstrated the encapsulation of both negatively and noncharged macromolecular cargoes within PMA HCs with a low number of deposited polymer layers. We also provide direct evidence for the semipermeable nature of PMA HCs based on the charge of the solutes. With rational design of the capsule membrane, PMA HCs afford differential permeability and high retention capabilities toward diverse cargo. These findings are of importance for future development and application of PMA HCs as microreactors.

Acknowledgment. This work was supported by the Australian Research Council under the Federation Fellowship and Discovery Project schemes.

Supporting Information Available: Encapsulation efficiency of PMA-loaded HCs cross-linked with varying concentrations of DTDP. This material is available free of charge via the Internet at http://pubs.acs.org.