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One-Step Microfluidic Fabrication of Polyelectrolyte Microcapsules in Aqueous Conditions for Protein Release

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Abstract: *We report a microfluidic approach for one-step fabrication of polyelectrolyte microcapsules in aqueous conditions. Using two immiscible aqueous polymer solutions, we generate transient water-in-water-in-water double emulsion droplets and use them as templates to fabricate polyelectrolyte microcapsules. The capsule shell is formed by the complexation of oppositely charged polyelectrolytes at the immiscible interface. We find that attractive electrostatic interactions can significantly prolong the release of charged molecules. Moreover, we demonstrate the application of these microcapsules in encapsulation and release of proteins without impairing their biological activities. Our platform should benefit a wide range of applications that require encapsulation and sustained release of molecules in aqueous environments.*

Polyelectrolyte (PE) microcapsules are aqueous droplets surrounded by a shell that is solidified owing to the electrostatic attraction between oppositely charged polymers rather than by crosslinking polymers using chemical reactions.[1] As such, they are widely used for encapsulation and release of chemically sensitive reagents in the pharmaceutical and food industries.[2] The classical way to fabricate PE microcapsules, however, requires solid particles as templates; this method uses layer-by-layer assembly to alternatively deposit oppositely charged polyelectrolytes onto the surface of a micrometer-scale particle and subsequently decomposes the template material into individual molecules that can pass through the shell.[3] The process of decomposition, however, often impairs the shell integrity and thus results in non-sustained release of the encapsulated contents.[4] Alternatively, PE microcapsules can be fabricated using emulsions, rather than solid particles, as templates. For example, using water-in-oil emulsions generated by microfluidic techniques, PE microcapsules have been successfully fabricated based on the complexation of oppositely charged polyelectrolytes at the water/oil interface.[5] Despite the promise of this emulsion-based technique, its use is severely limited because of the requirements of using organic solvents. Avoiding these solvents would greatly advance the application of PE microcapsules in encapsulating chemically sensitive biomolecules such as proteins; however, there remains an unmet need for the development of a simple method that enables the fabrication of PE microcapsules in biologically friendly aqueous conditions for protein release.

Herein, we report a one-step microfluidic fabrication of PE microcapsules in aqueous conditions. Unlike traditional methods that rely on organic–aqueous systems, we use two aqueous polymer solutions with a relatively small interfacial tension, yet large enough to generate transient water-in-water-in-water (W/W/W) double emulsion drops.[6] We use these transient W/W/W drops as templates to fabricate PE microcapsules based on the interfacial complexation of oppositely charged polyelectrolytes. Remarkably, the shell thickness of PE microcapsules is nearly independent of the concentration and molecular weight (MW) of the polyelectrolytes. We quantify the release profile of the PE microcapsules for both neutral and charged molecules. Interestingly, the release of negatively charged molecules is significantly prolonged because of attractive electrostatic interactions. Finally, we demonstrate the application of PE microcapsules in encapsulation and release of platelet-derived growth factor-BB (PDGF-BB) without impairing its biological activities.

We use a glass capillary microfluidic device to generate transient W/W/W emulsion drops that serve as templates for the formation of microcapsules (Supporting Information, Materials and Methods).[7] The device consists of two tapered cylindrical capillaries that are inserted into opposite ends of a square capillary, as illustrated by Figure 1 a. We use the left capillary to inject the inner phase consisting of 15 wt% dextran and 0.5 wt% poly(diallyldimethyl ammonium chloride) (PDDA), a positively charged polyelectrolyte (PE+). The middle phase, 17 wt% polyethylene glycol (PEG), is injected from the left through the interstices between the left cylindrical and the square capillaries. The outer phase is injected from the right interstices; it is essentially the same

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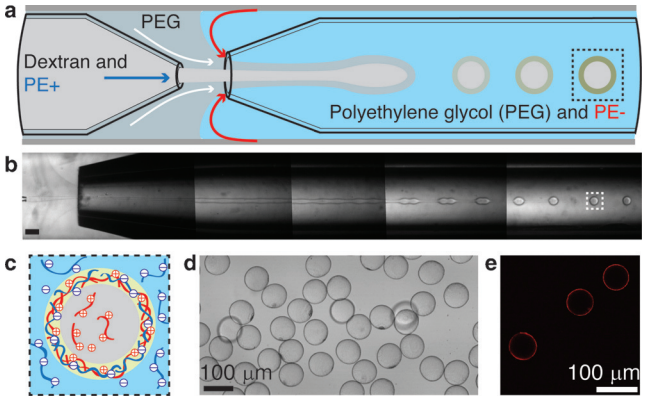

Figure 1. Microfluidic fabrication of polyelectrolyte capsules in aqueous conditions. a) Schematic of a glass capillary microfluidic device for generating water-in-water emulsions. Rings on the right side represent polyelectrolyte capsules. The rightmost outlined ring with darker color corresponds to a solidified capsule. b) Image of water-in-water-in-water (W/W/W) double emulsions formed in the glass capillary microfluidic device. c) Illustration of the solidification of a microcapsule due to the complexation of oppositely charged polyelectrolytes, PE+ (red lines) and PE− (blue lines), at the interface of a transient dextran/PEG/PEG W/W/W emulsion. d) A representative image of polyelectrolyte microcapsules. e) A confocal microscope image of polyelectrolyte capsules with the shell labeled by ethidium homodimer. Scale bar = 100 μm.

PEG solution as the middle phase, but contains 1.0 wt% polystyrene sodium sulfate (PSS), a negatively charged polyelectrolyte (PE−). The dextran and PEG solutions are immiscible, yet have a very small interfacial tension, about 0.1 mN m^{-1}, which is nevertheless large enough to allow the stable formation of monodisperse emulsions in a jetting regime, as shown by the image in Figure 1b and the Supporting Information, Movies 1 and 2.[6a,8] After the generation of a droplet, the PE+ molecules in its inner core diffuse towards the middle phase, where they meet the PE− molecules from the continuous phase to form a solid-like polyelectrolyte complex surrounding the drop. This process results in the formation of PE microcapsules, as illustrated in Figure 1c. The diameter of microcapsules is approximately 80 μm (Figure 1d), which is consistent with the estimate for the size of droplets formed in a jetting regime (see the Supporting Information).[8c] Moreover, the microcapsules have a uniform thin shell, as indicated by red rings in Figure 1e.

The production of PE microcapsules would not be possible without the middle aqueous phase, although it remains possible to generate single water-in-water droplets.[9] Although the middle phase is essentially the same as the outer one, except that the former does not contain polyelectrolytes, it is the middle phase that separates the oppositely charged polyelectrolytes prior to the formation of droplets. This transient separation avoids the complexation of oppositely charged polyelectrolytes at the opening of the capillary channels, and therefore, prevents the microfluidic device from clogging; consequently, the transient separation ensures a continuous production of transient W/W/W drops that serve as templates for the generation of PE microcapsules. However, unlike solid templating particles, the droplets do not have to be eventually removed.

We examined the morphology of PE microcapsules by scanning electron microscopy (SEM) (Supporting Information, Materials and Methods). Similar to typical hollow microcapsules, the particles buckle or collapse after drying, as shown in Figure 2a. Because the shell thickness is an

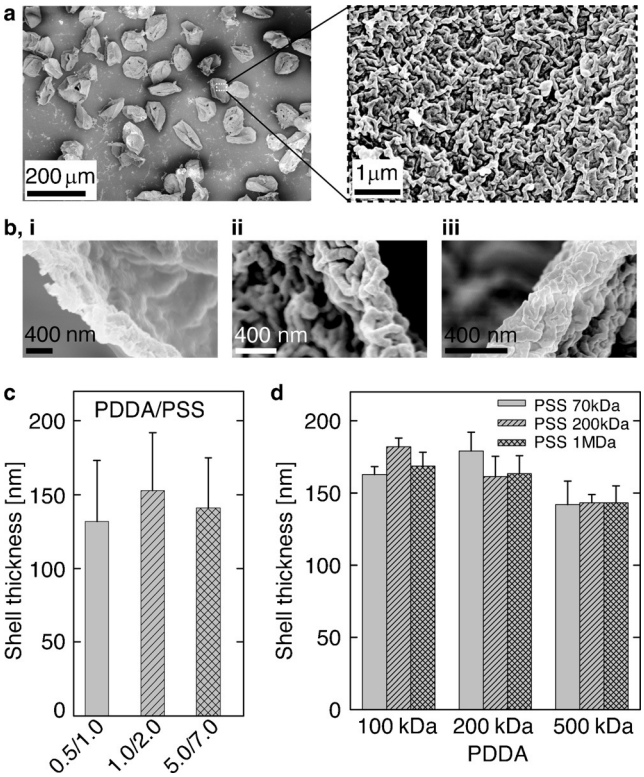

Figure 2. Morphology and shell thickness. a) A scanning electron microscopy (SEM) image showing that the polyelectrolyte capsules have a hollow structure. The concentration of PE+ is 0.5 wt% and for PE is 1.0 wt%. Inset: Morphology of the surface of a polyelectrolyte microcapsule. b) SEM images showing the cross-section of capsules formed by polyelectrolytes of different molecular weight with PDDA/PSS of (i) 100 kDa/70 kDa, (ii) 100 kDa/200 kDa, and (iii) 100 kDa/1MDa. c) Dependence of shell thickness on the concentration of polyelectrolytes with PDDA/PSS of 0.5:1.0, 1.0:2.0, and 5.0:7.0 (wt%:wt%). d) Summary plot of the shell thickness for nine combinations of polyelectrolyte molecular weight.

important physical parameter of microcapsules,[10] we next explored the possibility to tune it by varying the concentration of the polyelectrolytes. To quantify the shell thickness, we used SEM to examine the cross-section of microcapsules; examples are shown in Figure 2b. Surprisingly, the shell thickness was nearly constant with a value of approximately 150 nm, as the concentration was varied from 0.5 to 7.0 wt% (Figure 2c). This independence of the shell thickness on polyelectrolyte concentration is consistent with previous reports on PE microcapsules fabricated using water-in-oil emulsions as templates,[5a] but in striking contrast to microcapsules generated by conventional double-emulsion templating methods, in which the shell is formed by chemically

crosslinking polymers and the shell thickness increases with the polymer concentration.[11] The independence of the shell thickness on polyelectrolyte concentration is likely because cross-shell diffusion of the polyelectrolytes is required for the growth of the shell. This cross-shell diffusion, however, is severely hampered after the formation of a solid shell, as the polyelectrolytes not only experience the steric hindrance from the porous shell but are also subjected to attractive electrostatic interactions.[12] Consequently, the shell thickness of the microcapsules is nearly independent of the concentration of the polyelectrolytes.

While the shell poses a physical barrier for the diffusion of the polyelectrolytes, it remains possible for small polyelectrolytes to diffuse across the shell, which is effectively a porous membrane. Indeed, the dried PE microcapsules exhibit a rough surface texture that is reminiscent of a porous polyelectrolyte film (inset of Figure 2a).[13] Because cross-shell diffusion would result in an increase of shell thickness, we expected that the thickness could be tuned by changing the MW of the polyelectrolytes. Surprisingly, as their MW was varied from 70 to 1000 kDa, the shell thickness was nearly the same, approximately 150 nm (Figure 2d). This suggests that the shell of the microcapsules prevents the cross-membrane diffusion of the polyelectrolytes with a MW higher than 70 kDa.

The release of encapsulated molecules is determined by their capability to diffuse through the capsule shell. The release kinetics, therefore, can be affected by the steric hindrance of the pores in the shell. As such, polymers of higher MW should exhibit slower release, as they are subjected to stronger steric hindrance. As expected, the release of fluorescently labeled neutral dextran molecules becomes noticeably prolonged as their MW increases, as shown by the time-lapse fluorescence images in Figure 3a and the release profiles (empty symbols) in Figure 3b. To characterize the release rate, we calculated the characteristic release time, τ_{half}, at which the average fluorescence intensity of the microcapsules is half of its initial value. The τ_{half} increased from 10 to 100 min as the dextran MW was increased from 5 to 20 kDa (empty symbols in Figure 3c). Interestingly, as the MW of dextran was increased by a further factor of two to 40 kDa, τ_{half} increased by a factor of 10 to approximately 1000 min. Such a dramatic increase of τ_{half} indicates that the molecular weight cut-off of the porous capsule shell is lower than 40 kDa; above this value the majority of the molecules can no longer pass through the shell.[14]

Unlike neutral molecules, charged molecules can electrostatically interact with the polyelectrolytes, and such an interaction should alter their release kinetics.[13] Interestingly, fluorescein, a small molecule with MW of 332 Da and a negative charge owing to its carboxyl group, exhibited a sustained release, with $\tau_{half} \approx 600$ min. This slow release is in striking contrast to acridine orange, a molecule with a MW of 265 Da, yet positively charged. This molecule was released immediately after encapsulation, at a rate faster than we can measure, as shown by the nearly complete absence of fluorescence of the PE microcapsules immediately after collection (Supporting Information, Figure S1). These results

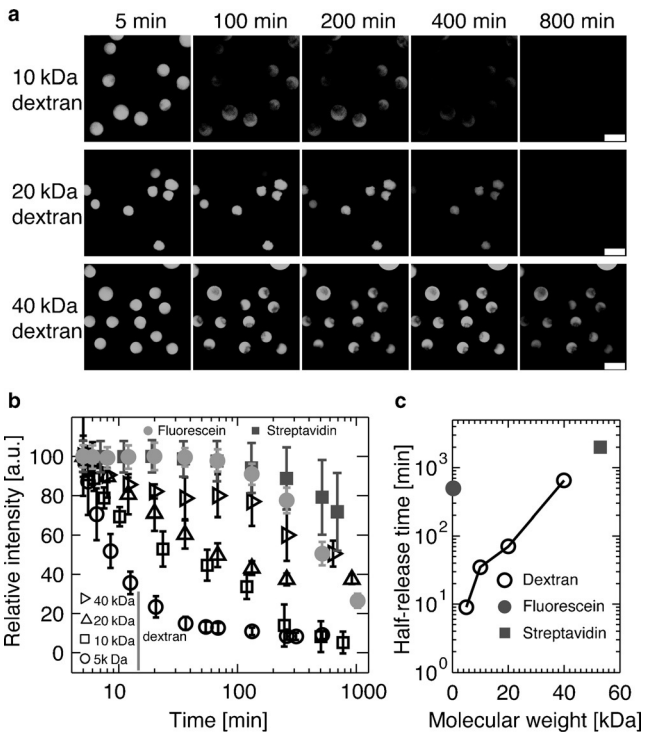

Figure 3. Release profiles. a) Time-lapse confocal microscope images showing the fluorescence of microcapsules encapsulated with neutral FITC-dextran molecules of different molecular weights and the protein streptavidin. Scale bar = 100 μm. b) Release profiles of FITC-streptavidin, fluorescein, and FITC-dextran with different molecular weights ranging from 5 to 40 kDa. c) Half-release time of neutral dextran molecules and charged molecules of streptavidin and fluorescein.

demonstrate that electrostatic interactions can dramatically alter the release rate of encapsulated molecules. Moreover, together with geometric constriction, attractive electrostatic interactions can be used to achieve an extremely long release for negatively charged proteins. For example, for microcapsules that encapsulate streptavidin, a negatively charged protein with a MW of 53 kDa and hydrodynamic radius of circa 5 nm, similar to the 40 kDa dextran, the fluorescence intensity decreased by only approximately 20% after 10 h (filled squares in Figure 3b). Moreover, we estimate that τ_{half} for streptavidin is about 10 days, based on extrapolation, nearly 20-fold longer than the 13 h measured for neutral 40 kDa dextran molecules. Collectively, our results demonstrate that attractive electrostatic interactions can significantly prolong the release profile of negatively charged molecules regardless of their molecular weight.

The PE microcapsules were fabricated under aqueous conditions without the use of any biologically hazardous chemicals; therefore, it should be possible to use these microcapsules to encapsulate and release chemically sensitive proteins without impairing their biological activity. To demonstrate this possibility, we encapsulated PDGF-BB, a protein that promotes cell growth and migration and is sensitive to organic solvents,[15] and tested its biological activity after release by measuring the sprouting of microtissues in a hydrogel after exposure to PE microcapsules that contained PDGF-BB.[16] After 20 h, these cells migrated

a much longer distance compared with the cells exposed to empty PE microcapsules. Moreover, the migration of cells in the presence of PE microcapsules was similar to the control cells, which were cultured in a medium with the same concentration of PDGF-BB, as shown by the phase-contrast images of microtissues in Figure 4a. To quantify the fraction

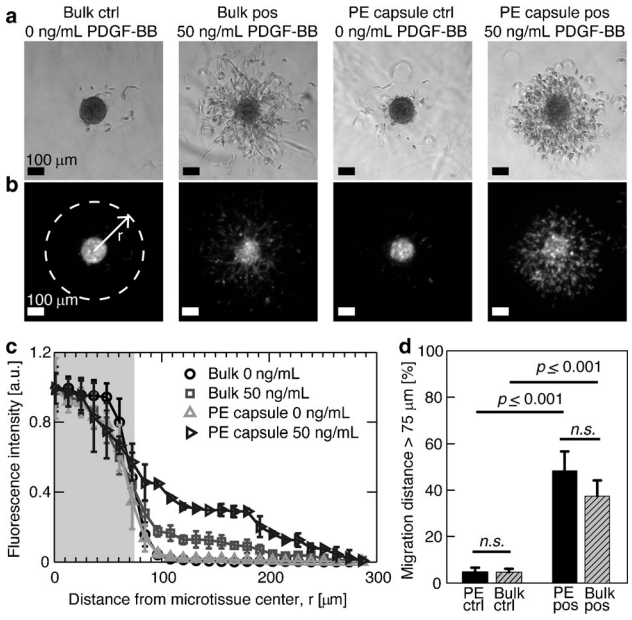

Figure 4. Bioactivity assay of proteins after release. a) Phase contrast images showing the sprouting of microtissues embedded in a polyethylene glycol (PEG) based hydrogel. The initial radius of microtissues is about 75 μm. From left to right: Microtissues cultured in cell culture medium without PDGF-BB, supplemented with 50 ng mL^{-1} PDGF-BB, loaded with empty PE microcapsules, and loaded with PE microcapsules with 50 ng mL^{-1} PDGF-BB encapsulated and released. Scale bar = 100 μm. b) Projections of *z*-stack fluorescence confocal microscopy images, taking into account the spatial transformation in spherical coordinates. c) Dependence of the radial distribution of fluorescence intensity from the center of the microtissues. The width of shadowed area represents the initial radius of the microtissues, circa 75 μm. d) Percentage of fluorescence intensity from distances greater than 75 μm from the center of the microtissue. Data is presented as mean ± SD, $n \geq 3$, n.s. = not significant.

of cells that left the initial region of a microtissue, we measured the fluorescence intensity from projections of *z*-stack fluorescence confocal microscopy images; examples are shown in Figure 4b (for details, see the Supporting Information, Materials and Methods section). For the cells cultured in medium containing PE microcapsules, the distribution of fluorescence intensity is similar to that for cells cultured in control medium (Figure 4c). Moreover, the migration of cells in the presence of PE microcapsules is in quantitative agreement with the controls in which cells were cultured in medium (Figure 4d). Thus, our measurements demonstrate that the PE microcapsules are cell-compatible and can be used to encapsulate and release proteins without impairing their biological activities.

In summary, we have developed a microfluidic approach that enables one-step fabrication of polyelectrolyte microcapsules in aqueous conditions. Unlike microcapsules formed by crosslinking polymers using chemical reactions, the shell thickness of microcapsules formed by the complexation of polyelectrolytes is nearly independent of both polymer concentration and molecular weight. Tunable shell thickness, however, should be possible by further depositing polyelectrolytes onto the surface of polyelectrolyte microcapsules using classic layer-by-layer assembly.[3a] Although a recent spraying technique offers a simple approach to generate PE microcapsules, it requires physiologically unfavorable conditions such as high salt concentrations and temperature.[17] The use of biologically friendly aqueous conditions is a unique feature of our platform and makes it valuable for encapsulating sensitive biomarkers. Additionally, the release profile of charged molecules can be tuned by harnessing electrostatic interactions. Moreover, it should also be possible to control the release using external stimuli such as pH or temperature by incorporating responsive polyelectrolytes into the capsule shell.[3c] Finally, the concept of fabricating polyelectrolyte microcapsules using transient W/W/W droplets as templates is not restricted to the types of charged polymers demonstrated here, but should also be applicable to other charged polymers with pre-engineered functionalities, such as motifs that can interact with cells.[3c] Combined with the versatility in choosing polyelectrolytes and its simple fabrication, our platform should benefit a wide range of applications requiring encapsulation and sustained release of molecules in aqueous environments.

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