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Dopamine-Mediated Continuous Assembly of Biodegradable Capsules

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Supporting Information

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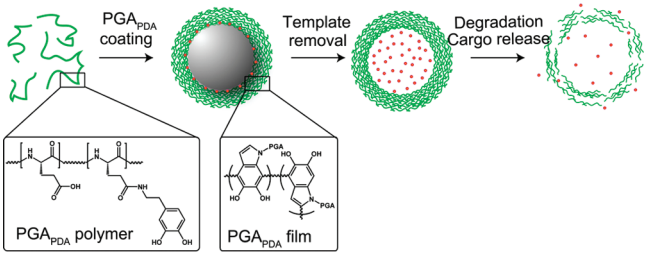
The assembly and utilization of nanoengineered films and particles is a rapidly growing area of research in the biomedical sciences. Over the past decade, the layer-by-layer (LbL) assembly approach has been widely used to produce capsules that are responsive to external stimuli,[1−4] degradable,[5−8] and amenable to the loading and release of therapeutics[9−15] and postfunctionalization for antibody targeting.[16,17] Recently we assembled poly(L-glutamic acid) (PGA)−drug conjugates into biodegradable capsules,[6,18] and showed that these drug-loaded capsules deliver drugs to cancer cells more effectively than the corresponding free polymer-drug conjugates.[6] Additionally, PGA-based capsules also circumvent P-glycoprotein-mediated multidrug resistance in colorectal cancer cells.[18] Although LbL capsules have potential for use in diverse applications, a limitation of the LbL process is the time-consuming and intensive assembly protocol.

Recently, Messersmith and co-workers pioneered the single-step formation of polymer films based on mussel-inspired polymerization of dopamine on various planar substrates.[19] This work has generated significant interest in polydopamine (PDA) coatings for a range of applications; both supported films, on electrodes[20,21] and carbon nanotubes,[22] and free-standing films have been assembled.[23] The dopamine- or norepinephrine-mediated formation of polymer films also provides a versatile platform for substrate-independent conjugation of biomolecules, proteins, and biodegradable polyester films.[24−26] Recent work has also focused on the assembly of PDA on spherical substrates.[27−31] Textor et al. used derivatives of dopamine conjugated to poly(ethylene glycol) chains to stabilize iron oxide nanoparticles.[30,31] We recently reported the formation of PDA capsules, obtained by polymerization of dopamin on particles of different sizes (between 0.5 and 5 μm) and composition (e.g., nonporous and mesoporous silica, emulsion droplets).[32,33] The PDA capsules did not significantly affect cell viability and were used to load hydrophobic cargos. However, controlled release systems often require tailored cargo release through biodegradation of the carriers. Although partial degradation of PDA films in vivo has recently been reported (in the order of weeks),[34] to the best of our knowledge, there have been no reports on the formation of tailored biodegradable particles/capsules mediated by the polymerization of dopamine.

Herein, we report the synthesis of dopamine-modified PGA-conjugates (PGA_{PDA}) for the continuous assembly of biodegradable capsules (Scheme 1). A range of PGA conjugates with different degrees of dopamine modification were synthesized and assembled onto silica particles. The PGA_{PDA} film/coating thickness was tailored via the dopamine content and the number of deposition steps. Stable PGA_{PDA} capsules were obtained after silica core removal. We demonstrate that the degradability of these capsules can be tuned through the percentage of dopamine within the films. Capsules fabricated using this approach combine the advantages of a biodegradable material with the one-step technique of dopamine assembly, thus offering significant advantages in the assembly of degradable capsules.

Scheme 1. Continuous Assembly of Dopamine-Modified PGA (PGA_{PDA} polymer) on Particles and Subsequent Removal of the Sacrificial Template, Yielding PGA_{PDA} Capsules (the capsules can be preloaded with cargo and degraded, resulting in cargo release)



PGA was modified with the dopamine monomer via amide bond formation to form PGA_{PDA} with various degrees of functionalization (7, 15, or 25%). These PGA_{PDA} polymers were then assembled from 10 mg mL^{-1} solutions (at pH 9) onto ~3 μm-diameter silica particles for 12 h. Upon centrifugation, the polymer-coated particles appeared brown/black. Nonsurface bound PGA_{PDA} was removed by several water washing/centrifugation cycles and the silica cores were dissolved to yield PGA_{PDA} capsules (see the Supporting Information for details).

The deposition of PGA_{PDA15} or PGA_{PDA25} onto the amine-modified silica particles through a single adsorption step, followed by core removal, resulted in the formation of stable capsules. In contrast, capsules were not obtained from PGA_{PDA7}.

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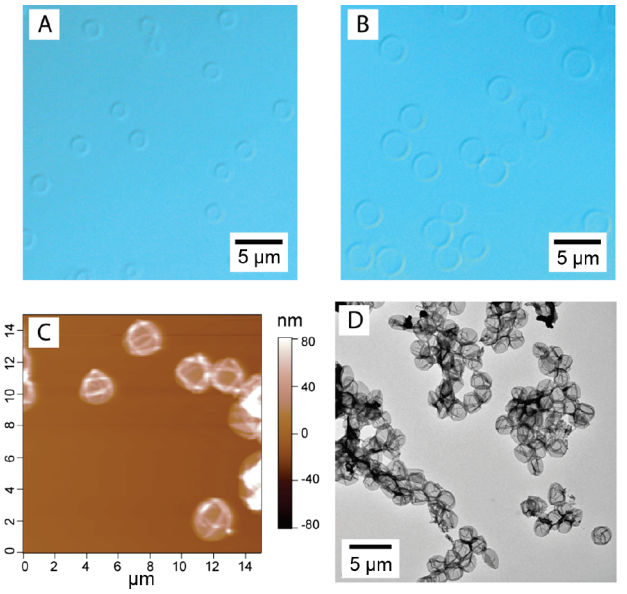

Figure 1. Differential interference contrast microscopy (DIC) images for (A) PGA_{PDA15} and (B) PGA_{PDA25} capsules. (C) AFM and (D) TEM images of PGA_{PDA25} capsules.

Table 1. PGA_{PDA} Capsule Wall Thicknesses (derived from AFM images)[a]

no. of deposition steps, D	PGA_{PDA7} (nm)	PGA_{PDA15} (nm)	PGA_{PDA25} (nm)
1	[b]	7.7 ± 1.2	14.7 ± 0.7
2	[b]	12.1 ± 0.7	[c]
3	[b]	20.8 ± 2.1	[c]
4	[b]	28.6 ± 2.6	[c]

[a] Values are the average of at least 10 capsules. [b] No capsules obtained. [c] Not measured.

Optical microscopy shows that capsules produced from PGA_{PDA15} exhibited significant shrinking (average capsule diameter = 1.6 μm, Figure 1A), compared with those obtained from PGA_{PDA25} (3.1 μm, Figure 1B). The larger diameter (and wall thickness, see Table 1) for the PGA_{PDA25} capsules is most likely due to the higher degree of cross-linking in PGA_{PDA25}. AFM (Figure 1C) and TEM (Figure 1D) images show that the capsules are collapsed, similar to what is observed for LbL polymer capsules.[35,36] The degree of PGA dopamine modification had a significant effect on the capsule wall thickness (Table 1). AFM profiles revealed single wall thicknesses of 7.7 and 14.7 nm for capsules assembled from the single-step deposition of PGA_{PDA15} and PGA_{PDA25}, respectively. Capsules prepared from pure PDA have wall thicknesses of approximately 20 nm.[32] The observed PDA-dependent film growth behavior suggests that the mechanism for PGA_{PDA} assembly is dopamine-mediated polymerization. Compared with other PGA capsules,[5,6,18] PGA_{PDA} capsules did not exhibit pH-responsive behavior. This is attributed to the high degree of cross-linking between dopamine groups, which also impacts on capsule degradability (see later).

Repeated exposure of PGA_{PDA}-coated silica particles to PGA_{PDA}-containing solutions results in thicker coatings/films, as evidenced by the larger capsule wall thicknesses (see PGA_{PDA15}, Table 1). Multiple deposition steps (one to four, denoted D1-D4) were employed (Table 1). This finding is consistent with our recent studies on PDA capsules.[32,33] No capsules were obtained from PGA_{PDA7} solutions, even after four deposition steps, suggesting that the degree of dopamine modification in PGA_{PDA7} is insufficient for film formation. Negligible shrinkage (compared with the template size) was observed for PGA_{PDA15} capsules formed after two deposition steps (D2), while PGA_{PDA25} capsules retained their size and shape after the first deposition step (D1). Microscopy analysis revealed negligible shrinkage and higher colloidal stability for capsules prepared with multiple deposition steps within a polymer series. Furthermore, for the same number of deposition steps, capsules obtained from polymers containing higher dopamine contents were more structurally stable; for example, PGA_{PDA15} (D4) capsules did not collapse when dried for AFM analysis—this is attributed to the increased capsule wall thickness (Table 1). Redeposition suggests dopamine-mediated polymerization is the dominant mechanism[19,24,25,37,38] for film growth, as pure PGA films do not self-assemble through multiple exposures.[5]

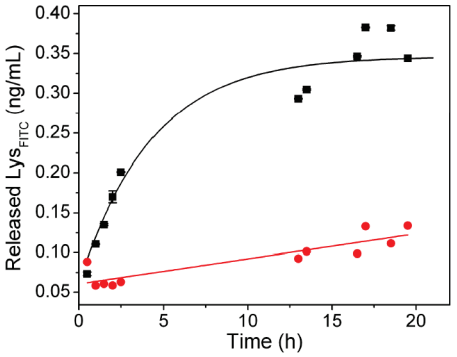

Figure 2. Lys_{FITC} release from PGA_{PDA15} (D3) capsules after incubation with protease (black squares), as monitored by fluorescence spectrophotometry. Control capsules (red circles) were incubated in the absence of protease.

Capsules obtained from three deposition steps with PGA_{PDA15} (D3) were exposed to protease solution to investigate their degradation behavior. Capsules were preloaded with fluorescently labeled lysozyme (Lys_{FITC}) (see the Supporting Information), and degradation was monitored via the release of the enzyme into the supernatant. As shown in Figure 2, Lys_{FITC} release reaches a plateau after ~15 h. This is attributed to degradation of the PGA, which allows diffusion of the encapsulated cargo into the surrounding medium. Only low levels of supernatant fluorescence and considerably slower leakage were observed in the absence of protease (Figure 2). The observed scattering in both series after 12 h is attributed to the increasingly difficult centrifugation behavior of the capsules due to their partial degradation.

To confirm that PGA degradation occurs, we performed quartz crystal microgravimetry (QCM) and atomic force microscopy (AFM) experiments on PGA_{PDA} films assembled under similar conditions on planar supports. PGA_{PDA15} D3 films showed an increase in QCM frequency with time after exposure to protease (up to ca. 12 h), indicating that these films degraded (see Figure S1 in the Supporting Information).[39] These data are in qualitative agreement with the enzyme release data for the PGA_{PDA15} capsules (Figure 2). In contrast, the QCM data indicate that D3 films made from PGA_{PDA25} do not degrade significantly.

AFM was used to analyze the morphology of the films. PGA_{PDA25} D2-D3 films exhibited a grainy surface (see Figure S2A,B in the Supporting Information), while the same films exposed to protease solution are smoother overall but pitted (depth of 3−4 nm) (see Figure S2,D in the Supporting Information), suggesting some PGA degradation. Furthermore, the number and size of "holes" were greater for PGA_{PDA25} D3 films (compared with D2 films; see Figure S2C and S2D in the Supporting Information). However, the AFM thicknesses for the PGA_{PDA25} films were the same (within error) before and after protease degradation, suggesting largely surface morphology changes (data not shown).

In conclusion, PGA conjugates with different degrees of dopamine modification were synthesized and assembled on particles, and biodegradable capsules were obtained. The thickness of the PGA_{PDA} coatings increased as a function of dopamine content and the number of deposition steps (for PGA_{PDA15}). The degradation kinetics were correlated with the dopamine content of the polymers. Exposure of the PGA_{PDA15} capsules to protease solution resulted in release of encapsulated lysozyme. Protease-induced degradation was also observed for PGA_{PDA15} films on planar supports, whereas negligible degradation was observed for PGA_{PDA25} films. The ability to tune the formation of biodegradable polymer coatings through continuous dopamine-mediated assembly is expected to lead to capsules (and films) with potential applications in biomedicine.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Details of the synthesis of PGA_{PDA}, assembly and cleaning procedures, instrumental methods, and QCM and AFM film degradation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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