Dopamine-Mediated Continuous Assembly of Biodegradable Capsules

*Christopher J. Ochs, Tam Hong, Georgina K. Such, Jiwei Cui, Almar Postma, and Frank Caruso**

[*] Prof. F. Caruso, C. J. Ochs, T. Hong, Dr. G. K. Such, Dr. J. Cui, Dr. A. Postma
Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010 (Australia)
E-mail: fcaruso@unimelb.edu.au

Dr. A. Postma
CSIRO Materials Science and Engineering, Clayton, Victoria 3168 (Australia)

Materials and methods

Poly(L-glutamic acid, sodium salt) (PGA, Mw = 50 000-70 000 Da), dopamine hydrochloride, hydrofluoric acid (HF), ammonium fluoride (NH_4F), and protease (from Streptomyces griseus, 4.6 units mg^{-1} solid) were purchased from Sigma-Aldrich. Amine-modified silica particles (5 wt% suspensions, average diameter 3.25 ± 0.18 μm) were obtained from Microparticles GmbH (Berlin, Germany). Unless specified otherwise, all other chemicals were purchased from Sigma-Aldrich and used as received. 10 mM tris(hydroxymethyl)-aminomethane (Tris) buffer (pH 9) was used for all polymerization experiments. The pH of solutions was measured with a Mettler-Toledo MP220 pH meter. High-purity water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore RiOs/Origin water purification system. Silicon wafers were obtained from MMRC Pty Ltd (Melbourne, Australia).

Synthesis of dopamine-modified poly(*L*-glutamic acid) (PGA_{PDA})

Poly(*L*-glutamic acid) (PGA) was modified with dopamine hydrochloride by dissolving 50 mg (0.23 mmol, 1 equiv.) of PGA in 4 mL of 50 mM sodium acetate buffer adjusted to pH 6. 100 mg (0.36 mmol, 1.5 equiv.) of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and dopamine in varying amounts were then added. The clear solution was stirred at room temperature for 2 h, purified on a Sephadex gel column and lyophilized to give a white powder. NMR analysis was used to determine the degree of modification with dopamine by comparing the integral of the aromatic dopamine signals with the integral value obtained for the α-hydrogen of PGA (set as 100%). The following PGA_{PDA} conjugates were synthesized (subscripts indicate the degree of dopamine modification in %): PGA_{PDA7}, PGA_{PDA15} and PGA_{PDA25}. ^1H NMR (400 MHz, D_2O, δ_H): 1.82-2.16 and 2.18-2.46 (*m*, 4H, PGA), 3.14-3.26 (*br s*, 2H, dopamine), 3.36-3.54 (*br s*, 2H, dopamine), 4.25-4.42 (*br s*, $1H_\alpha$, PGA), 6.95-7.24 (*br s*, 3H, dopamine) ppm.

Preparation of PGA_{PDA} capsules

The PGA_{PDA} polymers (PGA_{PDA7}, PGA_{PDA15} or PGA_{PDA25}) were assembled onto 3.25 μm-diameter silica particles from buffered (Tris) 10 mg mL^{-1} solutions at pH 9 for 12 h. The polymer-coated particles were then centrifuged and the supernatant exchanged with water several times to remove non-surface bound PGA_{PDA}. The above process was repeated for multiple depositions. The silica cores were then dissolved using 2 M HF/8 M NH_4F buffer (pH 5), and after centrifugation/ water washing cycles, PGA_{PDA} capsules were obtained. Our previous study on PDA coatings formed from 2 mg mL^{-1} solutions showed that 12 h was sufficient to achieve saturation coverage.[1] A higher concentration of PGA_{PDA} was chosen in the current study to account for the lower total dopamine content in the polymerization solution (compared to that used for pure PDA).

Preparation and degradation of lysozyme-loaded PGA_{PDA} capsules

Fluorescently labeled lysozyme (Lys_{FITC}) was loaded to PGA_{PDA} capsules using the following procedure. 150 μL of 3.25 μm diameter silica particles were washed into Tris buffer via three centrifugation/redispersion cycles and then exposed to 600 μL of 0.5 mg mL^{-1} Lys_{FITC} in Tris buffer (150 mM) for 15 min. After washing with Tris buffer, the particles were exposed to a solution of poly(methacrylic acid) (PMA, M_w= 15 000 Da, 1 mg mL^{-1}) for 15 min. This cycle was repeated and terminated by a final third layer of Lys_{FITC} to ensure sufficient loading with the fluorescent enzyme. The samples were then incubated with 600 μL of 10 mg mL^{-1} PGA_{PDA15} overnight, and then exposed to PGA_{PDA15} twice more. The sacrificial template was then removed using buffered HF to obtain Lys_{FITC}-loaded PGA_{PDA15} (D3) capsules. For degradation, the capsules were counted on a flow cytometer, and 700 000 capsules per sample were exposed to 600 μL of protease solution (10 mg mL^{-1} in (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer, 20 mM, pH 7.2) or HEPES buffer (control sample) at 37 ^{o}C. To monitor the progress of degradation, samples were spun down (3000 *g*, 4 min) and 200 μL of the supernatant solution was analyzed with fluorescence spectrophotometry. The aliquot was then replaced into the sample, which was redispersed and incubated until the next measurement.

Cleaning of substrates for planar film deposition

Gold-coated 5 MHz AT-cut crystals were cleaned with Piranha solution (70/30 v/v sulfuric acid/hydrogen peroxide) for 20 min followed by extensive rinsing with water, and drying with nitrogen. Cleaning of silicon wafers for atomic force microscopy (AFM) experiments was performed by submerging slides in Piranha solution (70/30 v/v% sulfuric acid/hydrogen peroxide)

for 20 min and rinsing thoroughly with water. [*Caution! Piranha solution is highly corrosive. Extreme care should be taken when handling Piranha solution and only small quantities should be prepared.*] This process was repeated and the slides were sonicated in 50 v/v% isopropanol/water for 20 min. Afterwards, the slides were heated to 60 °C in RCA solution (5:1:1 water:hydrogen peroxide:ammonia solution) for 20 min. Finally, substrates were washed with water and dried under a stream of nitrogen.

Instrumental methods

Flow cytometry measurements were carried out on a Partec CyFlow Space (Partec GmbH, Germany) flow cytometer at an excitation wavelength of 488 nm. Data were analyzed according to the procedure outlined previously.[2] Differential interference contrast (DIC) and fluorescence images were taken on an inverted Olympus IX71 microscope equipped with a DIC slider (U-DICT, Olympus) with a 60× objective lens (Olympus UPFL20 / 0.5 NA, W.D. 1.6). A CCD camera was mounted on the left-hand port of the microscope. A tungsten lamp was used for DIC images. Fluorescence images were illuminated with an Hg arc lamp, using a UF1032 filter cube. QCM measurements were conducted using a QCM-D E4 device with four flow cells (Q-Sense AB, Västra, Frölunda, Sweden). PGA_{PDA15} and PGA_{PDA25} films were assembled through multiple *ex situ* deposition steps (D3) onto QCM crystals. The QCM frequency for each crystal was normalized to 0 Hz prior to the addition of protease. QCM frequencies were recorded with time for the degradation of PGA_{PDA15} films after exposure to buffered protease (10 mg mL^{-1}) at 37 °C. All frequency values quoted are for the third overtone. For AFM and transmission electron microscopy (TEM) measurements, 1 μL of a concentrated capsule solution was placed on a clean silicon wafer slide (or TEM grid) and allowed to dry. AFM scans were carried out with an MFP-3D Asylum Research instrument in AC mode using ultrasharp SiN gold-coated cantilevers (NT-MDT). TEM analysis was carried out with a Tecnai TF30 microscope operated at 200 kV.

References

[1] Postma, A.; Yan, Y.; Wang, Y.; Zelikin, A. N.; Tjipto, E.; Caruso, F. *Chem. Mater.* **2009**, *21*, 3042.

[2] Johnston, A. P. R.; Zelikin, A. N.; Lee, L.; Caruso, F. *Anal. Chem.* **2006**, *78*, 5913.

QCM degradation of PGA_{PDA} films

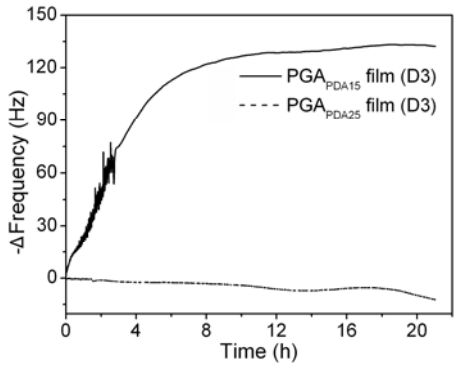

Figure S1. Degradation of PGA_{PDA15} and PGA_{PDA25} films after multiple depositions (D3) on QCM crystals. Films were exposed to 10 mg mL^{-1} protease at 37 °C and degradation was monitored by the change in frequency over time. Experiments were performed in triplicate. A majority of the error bars are within the size of the data point. No degradation was observed for samples incubated in the absence of protease.

Surface morphology of PGA_{PDA25} films deposited on planar templates before and after protease degradation

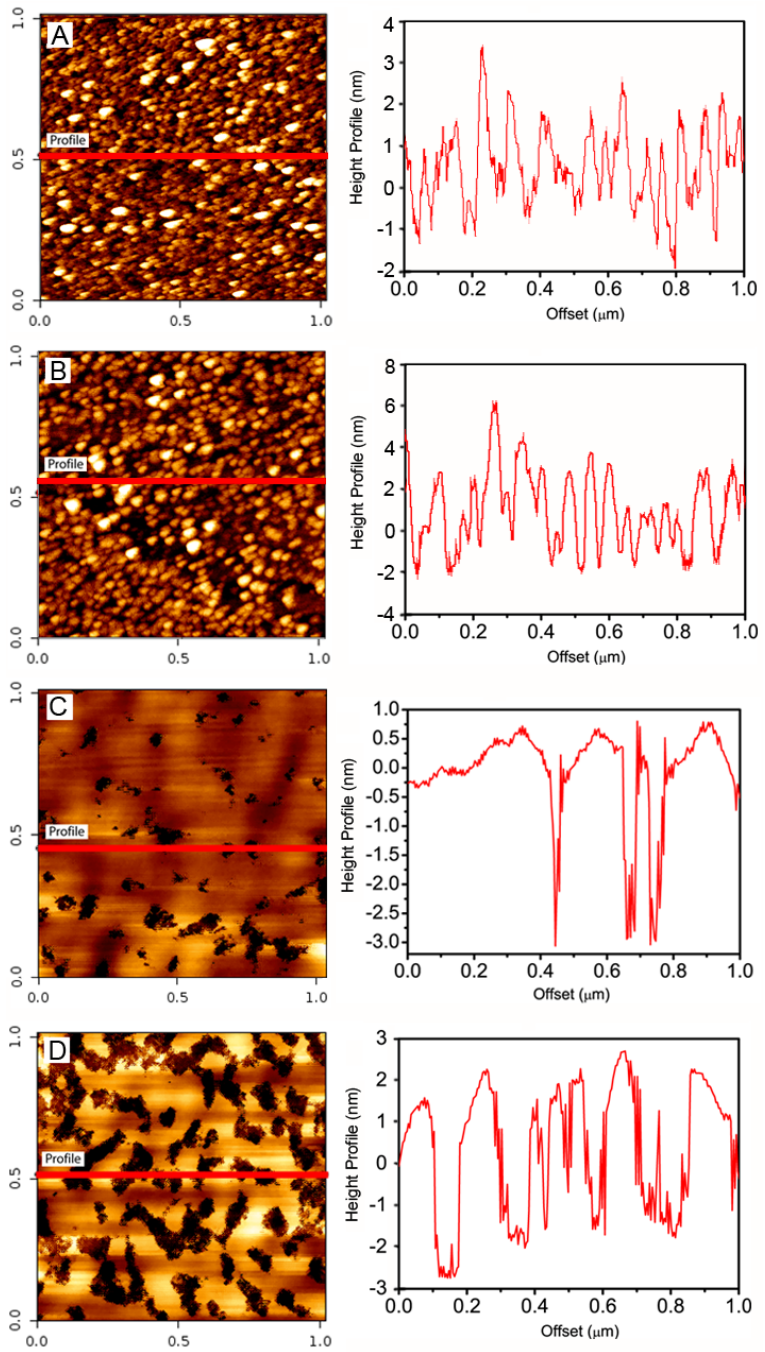

Figure S2. AFM images and height profiles of continuously assembled PGA_{PDA25} D2 (**A,C**) and D3 (**B,D**) films before (**A,B**) and after (**C,D**) enzymatic (protease) degradation. The films were assembled on planar substrates and some films (**C,D**) were subsequently exposed to a buffered protease solution (10 mg mL^{-1}) for 12 h at 37 °C. All films were rinsed with water and dried with nitrogen before AFM analysis.