Modified Multi-Arm Poly(Ethylene Glycol) Nanopatterns for Unidirectional Actin Polymerization

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Statement of Purpose: To date, protein immobilization at the nanoscale has been limited to two-dimensional patterns which are only nanoscale in height,[1] yet there is significant interest in the field of bionanotechnology to create three-dimensional structures for many bio-hybrid applications. In this study, we examined whether electron-beam (e-beam) crosslinked amine-terminated 8-arm poly(ethylene glycol) patterns could be used as a platform for unidirectional polymerization of actin. Actin is known to assembly into filaments up to several microns in length,[2] thus patterned surface initiated actin polymerization could result in biological nanostructures beginning to breach the third-dimension.

Methods: Freshly cleaned Si chips were immersed in 10 mg/mL poly(ethylene glycol) trimethoxysilane (Prochimia, Sopot, Poland) in anhydrous tetrahydrofuran (THF) and sonicated for one hour to passivated the background. Samples were rinsed with THF and Milli-Q H_20, and then dried with a stream of air. A 1% solution of 8-arm amine-terminated PEG (10,000 MW, Nektar, San Carlos, CA) in chlorobenzene was spincoated on the substrates. Crosslinked multi-arm PEG patterns were fabricated using a Leica EBL 100 e-beam lithography system (accelerating voltage: 71kV, current 40pA, dose: 100μC/cm^2 - 200μC/cm^2). Samples were rinsed with Milli-Q H_20 to remove any unreacted polymer and dried with a stream of air. Crosslinked PEG nanopatterns were visualized with a Multimode Nanoscope IIIA system (Digital Instruments, Santa Barbara, CA) operating in tapping mode. To confirm amine viability after e-beam exposure, samples were incubated with amine reactive Alexa Fluor 488 carboxylic acid, succinimidyl ester (5 mg/mL in dimethylformamide) for 30 min. Biotin was attached to the amine patterns by incubating with EZ-Link Sulfo-NHS-LC-Biotin (5 mg/mL in Milli-Q H_20, Pierce, Rockford, IL) for 30 min. Streptavidin was then immobilized by incubating the surface with 5 μg/mL in pH 7.4 PBS for 30 min. Biotinylated FX45 (recombinant gelsolin fragment) was mixed with F-actin at a ratio of 1:30 in 4 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 2 mM $MgCl_2$, 0.1 mM ethylene glycol tetraacetic acid (EGTA), 3 mM NaN_3, and 1 mM dithiothreitol (DTT) for 30 min to allow FX45 to sever F-actin and then cap the barbed ends of the short fragments. This complex served as the macroinitiator for surface initiated actin polymerization and was attached to streptavidin patterns by incubating (4 μM) for 1 hr, followed by washing with high Mg buffer (10 mM Tris (trishydroxymethylaminomethane), 0.2 mM ATP (adenosine 5'-triphosphate), 0.5 mM DTT, 0.2 mM $CaCl_2$, 0.02% NaN_3, 100 mM KCl and 5 mM $MgCl_2$ at pH 8.0). Fluorescent phalloidin labeled F-actin fragments were prepared by sonicating for a minimum of 15 min in a mixture of 1.6 μM F-actin and 0.375 μM Alexa Fluor 488 phalloidin. Surface initiated actin polymerization occurred by placing the sonicated F-actin on the substrate for 1 hr. All incubation steps were performed at room temperature.

Results/Discussion: Atomic force microscopy (AFM) images revealed crosslinked nanopatterns at locations that were exposed to the e-beam (Figure 1a). Incubation with the Alexa Fluor 488 carboxylic acid, succinimidyl ester resulted in green fluorescence specific to the patterned areas (Figure 1b), indicating the terminal amines were viable after crosslinking. Patterns below 200 nm are not visualized due to limits of conventional fluorescent microscopy. After immobilization of streptavidin and the macroinitiator complex, surface initiated polymerization of actin occurred, as indicated by bright green fluorescence over all patterned areas (Figure 1c). Preliminary confocal work with another patterned substrate demonstrated that patterns become difficult to image and blurring occurs once the actin filament are greater than 3-5 μm from the surface (not shown). As seen in Figure 1c, fluorescently labeled actin is visualized over patterned areas including those with sub-100 nm features; however, distinct patterns are difficult to visualize, thus indicating that the actin has formed free standing filaments of several microns in height.

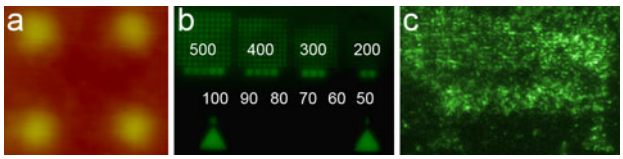

Figure 1. Amine-terminated PEG nanopatterns for actin polymerization. (a) AFM image of ~100 nm diameter crosslinked NH_2-terminated 8-arm PEG patterns. (b) Amine reactive dye bound to polymer nanopatterns. Diameter sizes are displayed in nm. (c) Image after actin was assembled on the nanopatterns.

Conclusions: Nanopatterns of amine-terminated 8-arm PEG were fabricated using e-beam lithography and served as a platform for surface initiated actin polymerization. Rather than simple two-dimensional protein patterns, high dimensional protein based nanostructures were demonstrated.

References:

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