A Novel, Photoswitchable Poly(ethylene glycol) Biosurface with Applications for High-Throughput Cell Migration Assays

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Introduction:

Mimicking *in vivo* conditions *in vitro* is of great importance to a wide range of disciplines. Biologists who wish to conduct more accurate studies of biological processes, tissue engineers who aspire to reconstruct organs, and pharmacologists who aim to better understand the effect of drugs on biological systems all depend on model systems that replicate the native microstructure of the human body. Various approaches have been developed to achieve this, including soft lithography and microfluidic devices. Novel biosurfaces that change cell adhesiveness via external stimuli (heat, voltage, light, etc.) – labeled "dynamic substrates" – have also attracted much attention as a viable option [1]. This summer, we investigated the latter, a novel photoswitchable poly(ethylene glycol) (PEG) biosurface that is responsive to UV light, which has also been tailored towards glass- bottom, 96-well plates. The surface shows promise for cell migration studies and other biological applications.

Material and Methods:

Surface Functionalization. Three types of photocleavable PEG polymers were synthesized as previously described [2]. In short, PEG-azides with molecular weights of 2000 Da, 5000 Da, and 11,000 Da were each combined with a photocleavable linker, 1-(5-methoxy-2-nitro-4-prop-2-ynyloxyphenyl)ethyl N-succinimidyl carbonate, in a 1 to 1.1 molar ratio under a copper catalyst and acetonitrile solvent. Compounds were purified by recrystallization and then analyzed with NMR.

Surface Functionalization. The functionalization of glass-bottom wells in 96-well plates required three steps. After washing with methanol, 50 μL of a 1 mg/mL poly-d-lysine (PDL) solution in 0.15 M NaCl was allowed to adsorb onto the glass surface. Following washing with H_2O, 50 μL of 1 mg/mL photocleavable PEG in 0.1 M sodium phosphate (pH = 7) and 0.6 M potassium sulfate was added to induce overnight PEGylation of the PDL surface. For mixed-chain tethered surfaces, PEGylation was repeated with a photocleavable PEG of lower molecular weight.

Photopatterning. A photomask was placed in the field diaphragm of an inverted microscope, and a mercury arc lamp exposed individual wells to a 10 J/cm^2 dose of UV light (λ = 365 nm) through a 10X objective.

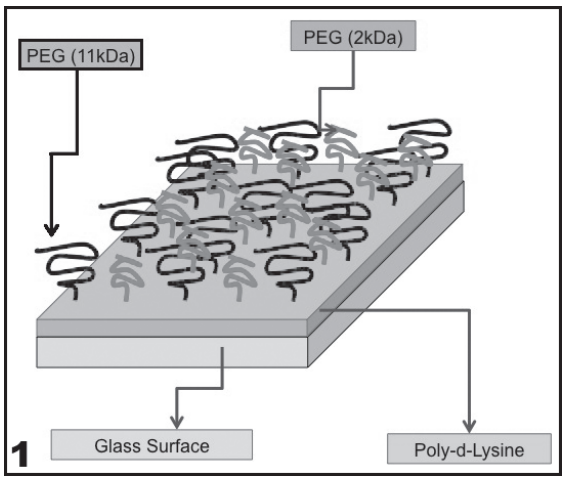
Cell Deposition. Madin-Darby canine kidney cells (MDCK) were deposited onto the photopatterned surfaces at 15,000 cells per well. One hour later, the surfaces were washed with medium three times. Cells were incubated overnight before further studies were conducted.

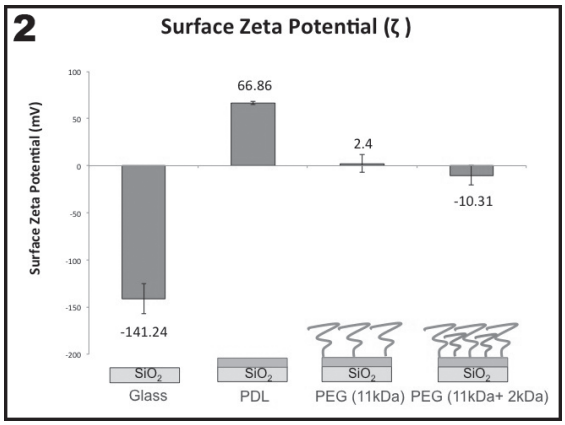
Cell Migration Studies. Various concentrations of cytochalasin d were added to cell-patterned wells. A flood exposure of 10 J/cm^2 initiated cell migration to previously uninhabited areas. Cell migration was monitored at approximately two-hour intervals and MetaMorph software was used to calculate change in cell-pattern area.

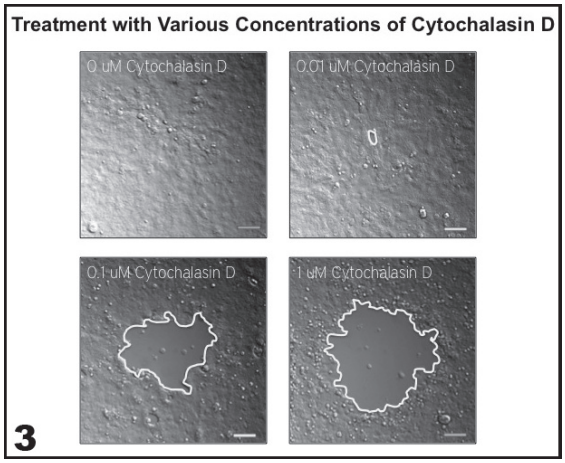
Results and Discussion:

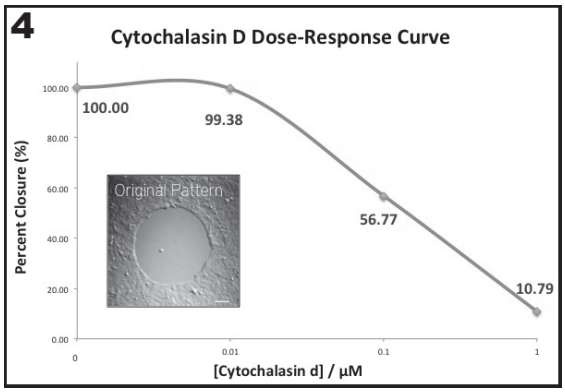
Our project focused on the functionalization, characterization, and application of a novel, photoswitchable surface to glass-bottom, 96-well plates, an inexpensive platform for high-throughput bioassays. The platform we investigated was a photocleavable, mixed-chain-tethered PEG surface grafted to PDL (PDL-g-PEG).

Both PEG and PDL have attracted much attention in literature. Because PDL is a cationic polyelectrolyte, it can easily adsorb onto negatively charged surfaces and is commonly used to enhance cell adhesion. On the other hand, PEG-tethered surfaces are anti-biofouling, where the effectiveness of repelling cells and proteins depends on the chain length and density of the PEG brush. However, a tradeoff exists between PEG length and brush density. In order to achieve both, a shorter PEG brush was introduced to a longer PEG-tethered surface. This mixed-chain-tethered PEG surface retained the long chain length while having increased density [3].









To transform this surface into a dynamic platform that switched from cell repelling to cell adhesive, the PEG chains were grafted to the PDL via the photocleavable linker molecule with a 2-nitrobenzyl group. Upon UV exposure, the 2-nitrobenzyl linker group was cleaved, releasing the mixed PEG brush and exposing the underlying PDL. Thus, irradiated areas switched from cell repelling to cell adhesive. This biosurface is visualized in Figure 1.

Three types of mixed-chain-tethered PEG surfaces grafted onto PDL were investigated: PDL-g-PEG (5kDa+2kDa), PDL-g-PEG (11kDa+2kDa), and PDL-g-PEG (11kDa+5kDa). Surface zeta potential measurements demonstrated the progressive functionalization of the glass surfaces. As shown in Figure 2, the inherently negative charge of the glass surface was altered to a largely positive charge upon adsorption of PDL. Grafting of the first PEG layer reduced the positive zeta potential from 66.86 mV to 2.4 mV. And the second PEGylation of the smaller chain brush reduced the potential further. These changes in zeta potential measurements suggest successful grafting of the PEG polymers to PDL.

The functionalized wells were then irradiated with UV using a photomask with a circular pattern. MDCK cells were seeded into these wells and allowed to populate on the photopatterned surface. Once cellular patterns were grown to confluence, cell migration tests were conducted. High-throughput screening of cytochalasin d, a cell migration inhibitor, was carried out. By testing a range of concentrations, a window of the dose- response curve was obtained, from which the half maximal inhibitory concentration (IC_{50}) could be derived. Figures 3 and 4 illustrate our preliminary findings.

In conclusion, we believe we have transformed a common piece of lab equipment into a novel, dynamic platform. While high-throughput drug screening of cell migration inhibitors has been demonstrated, we believe this easily functionalized surface has an extensive range of biological applications.

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References:

[1] Nakanishi et al., Analytical Sciences 24, 67-72 (2008).
[2] Kaneko et al. Phys. Chem. Chem. Phys 13, 4051-4059 (2011).
[3] Nagasaki, Y. Polymer Journal 1-10 (2011).

Figure 1: Visualization of PDL-g-PEG (11kDa+2kDa) biosurface.

Figure 2: Zeta potential shown for each step of PDL-g-PEG (11kDa+2kDa) functionalization.

Figure 3: Effect of cytochalasin d on MDCK circular-patterned areas on PDL-g-PEG (11kDa+2kDa) surfaces, observed 22 hours after flood exposure.

Figure 4: Cytochalasin d dose-response shown 22 hours after flood exposure of PDL-g-PEG (11kDa+2kDa) surfaces.