Photolabile micropatterned surfaces for cell capture and release†

Dong-Sik Shin, a Jeong Hyun Seo, a Julie L. Sutcliffe abc and Alexander Revzin a

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A method for capture and release of cells was developed using a photolabile linker and antibody-attached glass surface with a poly(ethylene glycol) (PEG)-pattern.

Surfaces are frequently micropatterned with proteins in order to capture and culture cells in distinct geometric configurations. Such micropatterned surfaces have important applications in cell-based diagnostics, tissue engineering and developmental biology. A variety of strategies for micropatterning cells on surfaces have been described including soft lithography, microfluidics, optical tweezers, inkjet printing and photolithography. However, it is often desirable to retrieve cells from micropatterns for more thorough downstream analysis. The methods for cell retrieval reported so far remain limited and may be broadly categorized into physicochemical, electrochemical or light-based. Physicochemical approaches include the use of shear or proteases to dislodge cells from protein coated surfaces. Other groups developed peptide-coated cell adhesive surfaces that could be rendered non-adhesive by chemical stimulation. Electrochemical approaches on the other hand may be used to locally stimulate cell release by applying voltage to individually addressable electrodes. While this approach has demonstrated merit, the need to culture cells on a conductive substrate and to fabricate electrode arrays for local cell release makes it somewhat complicated.

We hypothesized that T-cells expressing CD4 antigen could be captured inside the microwells and then released by UV-induced cleavage of Ab anchors. Here we describe the development of photolabile protein micropatterns for capture and light-triggered release of cells. In this approach, glass surfaces were modified using a mixture of amine- and acrylate-terminated methoxysilanes and then micropatterned using poly(ethylene glycol) (PEG) photolithography (see electronic supplementary information (ESI) for detailed description†). The mixed silane layer served a dual function: acyl groups helped to anchor PEG gel microwells and amine groups provided sites for covalent attachment of biomolecules within the microwells. These surfaces were further functionalized with azabenzotriazol-activated and 9-fluorenylmethoxycarbonyl (Fmoc)-protected photolabile linker containing photosensitive o-nitrobenzyl group (Scheme 1). Fmoc-groups were removed and photolabile molecules were then reacted with biotin-NHS (see ESI† for experimental detail). The micropatterned surfaces were further functionalized with avidin and biotin-anti-CD4 antibodies (Abs). This multi-step surface modification protocol resulted in formation of microwells with non-fouling walls and Ab-containing cell capture sites anchored to glass via photo-cleavable molecules.

Prior to carrying out cell capture and release experiments, we characterized release of avidin-biotin-photolabile linker construct from the surface. Two types of experiments, described diagrammatically in Scheme S1,† were carried out. In the first set of experiments, photolabile linker and biotin layers were assembled on amine/acryl functionalized glass, forming a uniform biomolecular layer. This surface was then exposed to UV light through a photomask so as to illuminate majority of the surface except for circular 100-μm diameter regions. Incubation of this surface with fluorescently-labeled streptavidin revealed much stronger fluorescence emanating from circular regions compared to background (~4:1 SNR, See Fig. 1A and C). This experiment demonstrated cleavage of biotin-photolabile linker constructs in the regions exposed to UV and retention of biotin in the circular region protected from UV light. In the data shown in Fig. 1A and C, surfaces were exposed to 365-nm UV at 1.2 W cm−2 for 0.5 s.

In the second set of experiments, we sought to characterize avidin detachment from photolabile surfaces. Direct exposure of surfaces containing fluorescently-labeled avidin was unsuccessful due to photobleaching. As an alternative strategy, mixed-silane containing surfaces were functionalized with photolabile linker, followed by biotin and neutravidin as described before (see Scheme S1† for details) and then exposed to 365-nm UV at 1.2 W cm−2 for 0.5 s through photomask containing 100-μm diameter darkfield regions. This treatment was expected to result in cleavage of the neutravidin-terminated biomolecular
layer. Incubation of this surface with fluorescently-labeled biotin revealed much stronger fluorescence signal in circular regions that were not illuminated by UV and were expected to retain neutravidin molecules (see Fig. 1B and D). However, the ratio of on the spot vs. off the spot fluorescence was 2:1 (Fig. 1D), suggesting some neutravidin molecules in the regions exposed to UV were still retained on the surface. In the future, molecules such as PEG or chitosan may be incorporated into the biointerface design to further minimize non-specific binding and to facilitated more efficient protein release upon UV activation. 15

In addition to micropatterning and fluorescence imaging, ellipsometry was employed to monitor the photocleavage of biomolecular constructs. These experiments were performed on silicon wafer pieces functionalized in the manner identical to that described for glass. Fig. 2A shows ellipsometry measurements performed at each step of the surface modification protocol. These results show a 7.2-nm thickness increased after silanization, suggesting presence of a multi-layer silane film. 16 Assembly of photolabile linker and biotin caused a further thickness increase of 1.4 nm, while attachment of neutravidin added another 3.0 nm to the multi-layer construct. The overall thickness of this photolabile biointerface was 11.5 nm before and 10.3 nm after UV exposure. This experiment points to partial removal of molecular from photolabile interface upon UV exposure. Plot of thickness change vs. time of UV exposure shown in Fig. 2B demonstrates that no appreciable cleavage was observed after 15 min by UV exposure at 1.8 mW cm−2 (Fig. 2B).

To prove the concept of UV triggered cell release, PEG microwell arrays containing photolabile avidin terminated attachment regions were functionalized with anti-CD4-biotin.
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Fig. 3 Cell populations in wells before and after UV exposure: (A) before UV exposure; (B) at 2 min UV exposure; (C) count of residing-in cells depending on UV exposure.

These microwells were then incubated with CD4 antigen expressing T-cells (Molt-3). Fig. 3A shows selective attachment of cells inside the microwells with minimal attachment occurring on non-fouling PEG hydrogel regions. Molt-3 cells are non-adherent CD4 expressing lymphoblasts that were captured inside the microwells solely due to the presence of anti-CD4 Ab. They did not spread on the surface but they were immobilized on the surface with antibody-cell interaction. Hydrogel microwells remained adherent on the surface for 5 to 7 days. Glass slides with cell arrays were placed in a Petri dish and exposed to 365-nm UV at 500 mW cm$^{-2}$. Cells started detaching immediately upon UV exposure and 90% of T-cells were detached after 1 min exposure followed by gentle rinsing (Fig. 3B and C). Importantly, Fig. S2† demonstrates that conditions used for cell release did not adversely affect cell viability.

In conclusion, we have developed micropatterned photolabile surfaces for capture and light-triggered release of cells. These surfaces consisted of microwell arrays with non-fouling PEG gel walls and cell adhesive photolabile glass bottom. Cleavage of biointerface components was characterized by fluorescence microscopy and ellipsometry. As a proof of concept demonstration, microwell arrays were functionalized with anti-CD4 Ab and were used to capture CD4-expressing T-cells (Molt-3 cells). A short exposure to UV was sufficient to cleave anchors holding cells to the surface, leading to cell release upon gentle agitation. Importantly, this cell retrieval protocol did not compromise cell viability. The biointerface design described here is an important addition to a limited repertoire of cell retrieval technologies reported in the literature. Unlike other light-triggered release technologies based on lasers, this approach utilizes standard UV sources available in most laboratories. In the future, we envision integrating this cell retrieval approach with single cell detection strategies under development in our lab$^{13,17}$ to enable release of specific groups of cells based on secreted signalling molecules.

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References


