The Fabrication of Surfaces Displaying Multiple Micropatterns of Different Adhesive Ligands

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Statement of Purpose: Surfaces displaying nano- and micropatterned cell adhesive ligands have provided a platform that has led to numerous discoveries in cell biology and laid a foundation for engineering cell behavior via cell-surface interactions.[1] Soft lithography techniques are well suited for creating surfaces displaying patterns of one ligand type in a single arrangement but are difficult to implement in the creation of multifaceted surfaces that present multiple ligand types each in their own defined patterns. To circumvent these limitations we have developed Laser Scanning Lithography (LSL). We demonstrate that LSL can be implemented to create surfaces displaying ligands patterned from the nano- to micrometer length scales in any arbitrary configuration without the need for photolithographic masters and that multiple ligands can be patterned with each ligand confined to its own pattern.

Methods: Piranha cleaned coverslips were coated with 2 nm of titanium and 8 nm of gold with an electron beam evaporator. After cleaning in TL1 solution, the surfaces were functionalized with a 2 mM ethanolic solution of oligo(ethylene glycol)-terminated alkanethiol for 1 hour. Desired regions of the OEG-alkanethiol were thermally desorbed using a Zeiss 5 Live confocal microscope equipped with a 532 nm laser operating at 4.11 mW with a 1.61 µsec pixel dwell time.[2] The desorbed regions were then backfilled with a 2 mM ethanolic solution of either hexadecanethiol (HDT), GRGDS-OEG-terminated alkanethiol, or biotin-OEG-terminated alkanethiol for 20 minutes. X-ray photoelectron spectroscopy (XPS), ellipsometry, and contact angle measurements were implemented for characterization. Surfaces backfilled with HDT were exposed to fibronectin (FN) at a concentration of 10 - 12.5 µg/mL in PBS for 20 minutes while those backfilled with biotin-OEG-terminated alkanethiol were exposed to fluorescently-labeled streptavidin. Surfaces exposed to FN were seeded with NIH 3T3 Fibroblasts (Fb) that were fixed, stained with DAPI and phalloidin, and imaged with fluorescent and scanning electron microscopy (SEM).

Results: We demonstrate that LSL is powerful surface patterning technique with the ability to create sub-diffraction limited features (Figure 1A) and that arbitrary patterns can easily be created without the need for photolithographic masters (Figures 1A,B). We also demonstrate bioactivity of these surfaces and show that Fb cultured on 1 by 8 µm FN ellipses aligned their F-actin stress fibers to the ellipses after only 7 hours (Figure 1C).

As a proof of principle for the creation of multifaceted surfaces, we show that OEG-terminated alkanethiols can be desorbed and backfilled with biotin-OEG-terminated alkanethiols. Cyclic repetition of this process with exposure to fluorescently labeled streptavidin between successive patterning steps allowed for independent imaging of the 1st and 2nd thiol backfills (Figure 2 A,B).

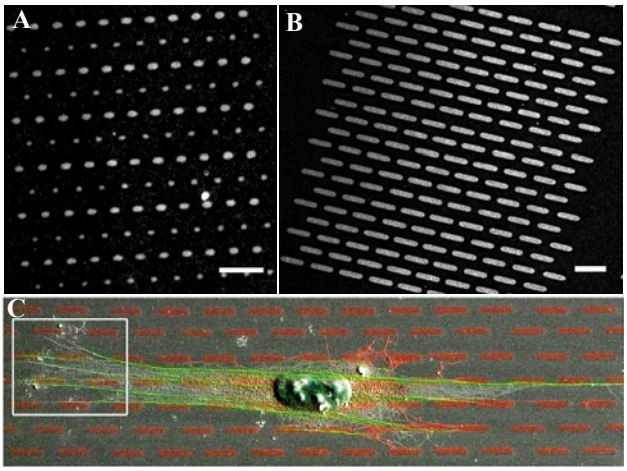

Figure 1: (A) Circular and oval patterns ranging in size from 0.17 to 0.68 µm^2 were created with a 532 nm laser and a 63X objective. SB = 5 µm. (B) Elliptical patterns, 1 by 8 µm, were created with a 532 nm laser and a 20X objective. SB = 10 µm. (C) Composite SEM and fluorescent image of a Fb cultured on surfaces shown in B. Red=FN, Green=F-actin, Blue=Nucleus. SB = 5 µm.

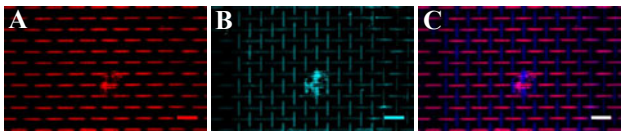

Figure 2: (A) OEG-terminated alkanethiols were desorbed from the surface, backfilled with biotin-terminated alkanethiols, and labeled with streptavidin-AF633. (B) A second cycle of desorption and biotin backfilling with streptavidin-AF568 led to some non-specific adsorption to the pattern in A. (C) Merge of A and B. SB = 10 µm.

After the 2nd backfill step, some non-specific adsorption to the 1st patterned region was observed (Figure 2B). This effect is due to drying of the samples between patterning cycles and we demonstrate that it can be minimized by performing protein adsorption in the last cycle. Using this approach we have fabricated surfaces displaying orthogonal patterns of GRGDS-OEG-terminated alkanthiol and FN for competitive cell alignment studies.

Conclusions: We have demonstrated that surface patterning of alkanethiol on gold using LSL has advantages over existing patterning strategies. These advantages include the ability to create arbitrary patterns "on the fly" without the need for photolithographic masters, the ability to integrate both nano- and micrometer size patterns in the same region, and most importantly the ability to pattern multiple ligands on the same surface with each ligand confined to its own pattern.

References:

1.Weibel DB. Nat Rev Microbiol. 2007;5:209-218.
2.Shadnam MR. J Phys Chem B. 2005;109: 11996-12002.