Laser Scanning Lithography and Soft Lithography of Micropatterned Cell-adhesive Self-assembled Monolayers

Jordan S. Miller[1], Mathilde Bethencourt[2], Mariah Hahn[1], T Randall Lee[2], and Jennifer L West[1]
[1] Department of Bioengineering, Rice University, Houston, TX, USA
[2] Department of Chemistry, University of Houston, Houston, TX, USA

Statement of Purpose: Soft lithography has recently shown promise in directing specific cell-substrate interactions. We have developed laser scanning lithography (LSL) with a commercially available confocal microscope utilizing virtual masks for the preparation of patterned glass masters for use in soft lithography. This convenient technique provides even exposure across the entire view field and facilitates accurate alignment of successive photoresist exposures. Photoresist masters prepared by laser scanning lithography enabled the successful patterning of hexaethylene glycol- and GRGDS-terminated self-assembled monolayers (SAMs) on gold, and provided for the adhesion and confinement of human dermal fibroblasts (HDFs) within the patterned areas.

Methods: *Laser Scanning Lithography*
Glass coverslips (Fisher Scientific; Pittsburgh, PA) were cleaned, primed, and spin-coated with S1813 photoresist (Shipley; Marlborough, MA) as previously described.[1] Virtual masks were drawn using the Region of Interest (ROI) function of a Zeiss LSM 510 META microscope. These regions were ablated with a 10x Plan-Apochromat air objective, numerical aperture 0.45, using 0.9 mW/μm^2 from a 458 nm argon ion laser line for 32 μs/μm^2. Differential interference contrast (DIC) imaging was accomplished using a non-destructive 514 nm argon ion laser line.

Soft Lithography and Cell Patterning
Poly(dimethylsiloxane) (PDMS) stamps were replica-molded from the lithographically-patterned glass masters using Sylgard 184 (Dow Corning; Midland MI). Deposition of a 100 Å gold layer on coverslips and synthesis of functionalized alkanethiols were performed as previously described, and verified by ellipsometry and NMR.[1,2] PDMS stamps were inked with an ethanolic solution of a non-adhesive hexaethylene glycol-terminated thioalkane (2 mM) and stamped onto the gold layer for 10-30 sec. The coverslips were then dipped in an ethanolic solution of a cell-adhesive GRGDS-terminated thioalkane (2 mM) for 2 min to fill in the unstamped regions of the gold surface with an adhesive moiety. HDFs were seeded onto stamped and unstamped coverslips at a density of 2.1 x 10^4 cells/cm^2 and cultured for 7 days.

Results / Discussion: Laser scanning lithography was successfully developed and used to create coverslip masters for use in soft lithography. DIC imaging was utilized with a non-destructive wavelength as an immediate quality check of the ablation as well as to align successive ablations (Figure 1). Three-micron features have been achieved with a 10x objective and are on the order of the laser beam width at the focal point.

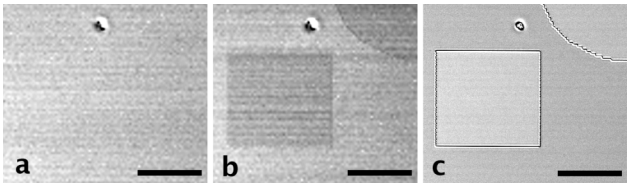

Figure 1. a) Fresh photoresist, b) DIC imaging after exposure, and c) developed master. Scale Bar = 50 μm.

Developed masters were successfully replica-molded into PDMS with high fidelity (Figure 2). HDF adhesion and spreading were limited to adhesive SAM areas (Figure 3).

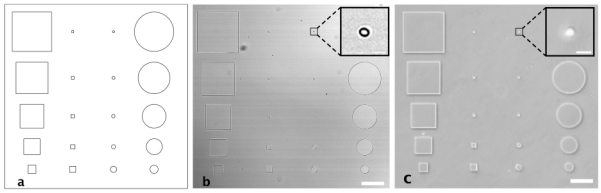

Figure 2. a) Virtual mask, b) developed master, and c) replica-molded PDMS stamp. Scale bar=100 μm. The minimum feature size achieved with a 10× objective (NA 0.45) was 3 μm (enlarged in b,c; scale bar=5 μm).

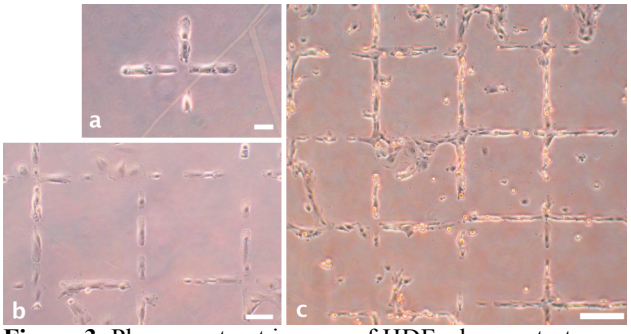

Figure 3. Phase contrast images of HDFs demonstrate their confinement within cell-adhesive regions. Scale bars: a=50 μm, b=100 μm, c=200 μm.

Conclusions: Laser scanning lithography of photoresist-coated glass masters enables micron-scale features to be generated with high fidelity and greater facility than conventional photomasking. DIC imaging permits immediate verification of each exposure, precise alignment of successive scans, and reduced chromatic aberration between imaging and exposure. This method rapidly produces masters for use in soft lithography, and was confirmed by HDF confinement within patterned SAMs.

References:
1. Heller DA, et al. Biomaterials. 2005; 26(8):883-9.
2. Roberts C, et al. JACS. 1998; 120(26):6548-55.

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