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One-post Patterning Multiple Protein Gradients by a Low-cost Flash Foam Stamp

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Supplementary Information

Materials

Photosensitive foam (polyethylene film) with a thickness of 4 mm, sulphuric acid tracing paper, transparent exposure film, and stamp machine were purchased from Hai Tian Electronic Information Materials Co., Ltd. (Shanghai, China). Cy3-labeled immunoglobin (IgG), (3-aminopropyl) triethoxysilane (APTES), glutaradehye (GA) were ordered from Sigma-Aldrich. Low melting point agarose was purchased from Genview Scientific Inc., China.

Human Umbilical Vein Endothelial Cells (HUVEC) cells were obtained from Dr. Yuan Li from Chongqing Medical Unvieristy. The cell was cultured in RPMI 1640 medium (Gibco) supplemented with 10% foetal calf serum (FCS, Gibco), 100 U/mL penicillin and 100 U/mL streptomycin at 37oC in a humidified 5% CO_2 incubator. FITC-labelled phalloidin and 2-(4-Amidino-phenyl)-6-indolecarb-amidinedihydro-chloride (DAPI) were purchased from Beyotime Biotechnology (Beijing, China). Collagen Type I (Rat Tail) were purchased from BD Bioscience.

All other chemicals were bought from Sigma-Aldrich and used without further purification unless otherwise indicated. All solution was prepared with deionized (DI) water produced by PURELAB flex system, ELGA Corporation

Fabrication of flash foam stamp

The desired structures were designed on the CorelDraw 10 (Corel Co., Canada) and printed on sulphuric acid tracing paper with a Xerox CP405d laser printer (Fuji Xerox, Japan). Then the mask paper, flash foam, and a piece of transparent exposure film were assembled from top to down and then exposed in a flash foam machine.

The light energy can be delivered by the stamp machine are: level 1- 260 Joule (J), level 2- 520 J, level 3-780 J, level 4-1040 J, level 5- 1300 J, level 6- 1560 J, level 7- 1820 J, level 8- 2080 J, level 9- 2340 J, level 10- 2600 J. In this study, to fabricate FFS from the mask paper with the same grey density (100% black), the energy level is 780 J and exposure time is 30 second. While to fabricate FFS with different grey density mask paper, the energy level is 1040 J and exposure time is 30 second.

Patterning of APTES, GA, protein and agarose

5% APTES ethanol solution (v/v), GA (0.5% v/v, diluted with DI H_2O), Cy3-labelled IgG (1:10000) and agarose (0.5% w/w, diluted by 0.9% NaCl) were used as inks.

APTES: FFS stamp was placed in the 5% APTES ethanol solution (v/v) for 5 min. The stamp surface was then carefully cleaned with an ethanol wipe. The stamp was pressed on NaOH (1 M) and oxygen plasma treatment hydroxylized glass slides. The patterned APTES-slide was treated with additional GA and protein.

GA: Oxygen plasma hydroxylized glass slides were immersed in an APTES/ethanol solution for 30 min and then dried with nitrogen. The GA ink was filled into the stamp by dropping the solution on the surface and soaking for 30 min. Then ink-filled stamp was firmly pressed on the APTES-slide. The Cy3-labelled protein was dropped onto the GA-slide and incubated for 30 min.

Protein: The hydroxylized glass slides were immersed in APTES/ethanol solution for 30 min and then dried with nitrogen. The APTES-slide were placed in a GA solution for 0.5 h and then washed with DI H_2O. The Cy3-labelled IgG (1:10000 dilution) were filled in the foam stamp by pipetting the protein solution onto the stamp with re-soaking for 2 h. The surface of the stamp was then thoroughly cleaned with a TBS-Tween 20 solution-wetted wiper. Finally, Cy3-labelled protein was printed onto the GA-glass slide.

Agarose: The 0.5 % (W/W) low melt point agarose was prepared with warm (45°C) 0.9% NaCl. The Cy3-labelled protein was mixed

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into the melted agarose and soaked into the warm stamp. The surface of the agarose-loaded stamp was carefully cleaned with a warm saline wipe. The protein-loaded agarose was then printed on a clean glass slide. All protein patterns on glass slides were visualized with a microarray scanner (LuxScanTM 10K, CapitalBio Corporation, China).

One-post patterning of protein gradient

Stamp fabrication: The structure on the mask was defined with different grey intensity values: 100% black, 80% black, 60% black, 40% black and transparent. The mask was then laminated on flash foam and irradiated with 1040 J incident light for 30 seconds.

Protein printing: Cy3-labelled IgG (1:10000 dilution) was filled in the foam stamp and printed on cleaned glass slides.

Cell patterning

Extracellular matrix Protein printing: Collagen (10 μg/mL) was filled in sterilized foam stamp and printed on cleaned glass slides.

Cell seeding and staining: HUVEC cells (1 X 10^5 cell/mL) in serum-free RPMI 1640 medium was placed on collagen-patterned slides and incubated at 37°C for 1 h. Then non-adherent cells were removed with gentle pipetting. The serum-free medium was replaced with RPMI 1640 plus 10% FCS. The residual adherent cells were cultured for 12 h and stained with FITC-labelled phalloidin and DAPI (Beyotime Biotechnology, China) to visualize the cell cytoskeleton and nuclei. In brief, cells were fixed in 4% paraformaldehyde in PBS at room temperature (25°C) for 5 min. The cells were gently washed twice with PBS, and treated by 1% Triton X-100 containing 1% BSA for 5 min. They were then incubated with FITC-phalloidin (5 μg/mL) at 37°C for 10 min. And then for nuclei staining, cells were mounted with DAPI solution for 5 min (5 ng/mL). Fluorescent dye probed cells were imaged with microscopy (Olympus IX71, Japan).