CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200900542

Supporting Information

for

Generation of Surface-Bound Multicomponent Protein Gradients

Kechun Zhang, Ayae Sugawara, and David A. Tirrell*

Cloning, expression and purification of proteins. Genes encoding the elastin mimetic domain ELF and leucine-zipper peptides ZE and ZR were constructed previously.[1] Because ZRELF is more soluble than the ZE(gs)$_6$ELF scaffold we reported in ref [1], we prefer ZRELF for protein immobilization. ZRELF containing *p*-azidophenylalanine was expressed in phenylalanine auxotrophic *E. coli* strain AF-IQ.[2] Gene fragments encoding FNZE and QKZE were ligated into pQE60 and the resulting plasmids were transformed into *E. coli* strain BL-21. Protein expression in 2XYT medium was induced by 1 mM IPTG at 22^{o}C. FNZE and QKZE were purified on Ni-NTA columns; purity of all expressed proteins was assessed by SDS-PAGE and MALDI-TOF mass spectrometry. Protein sequences are shown below:

ZRELF

MKGSLEIRAAALRRRNTALRTRVAELRQRVQRLRNEVSQYETRYGPLGGGGSGGGGSG[(VPGVG)$_2$VPG**F**G(VPGVG)$_2$]$_5$VPGC

FNZE

MKGSKKVSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTPRGDWNEGSKPISINYRTTSGSLEIEAAALEQENTALETEVAELEQEVQRLENIVSQYRTRYGPLGGGRSHHHHHH

QKZE

MKGSKLTWQELYQLKYKGIGGGGSGSLEIEAAALEQENTALETEVAELEQEVQRLENIVSQYRTRYGPLGGGRSHHHHHH

The ZR sequence is shown in green, ELF in red, Fn in orange, ZE in blue, and QK in purple.

Surface functionalization. Standard glass slides (Corning) were immersed in concentrated H_2SO_4 for 1 h. After washing thoroughly with water, they were immersed in a boiling solution of H_2O_2 (30%) / NH_4OH (30%) / H_2O (1:1:5 *v/v*) for 30 min and gently shaken in 1% octyltrichlorosilane in toluene for 3 h. Finally they were rinsed in MeOH (2x) and in deionized water (2x). Functionalized slides were cured at 110 ℃ for 30 min.

A solution of ZRELF (50 µL, 2.5 mg/mL) in 50% propan-1-ol was applied to a glass slide and spun at 1,500 rpm for 45 s. The resulting protein film was irradiated by Hg-arc lamps (I-line and H-line, 4.5 mW cm^{-2}) in a Karl Suss mask aligner for 2 min. Protein-coated slides were washed thoroughly with 50% isopropanol and doubly distilled H_2O to remove uncrosslinked protein.

Fabrication of microfluidic chips. The microfluidic gradient generator was fabricated by rapid prototyping and soft lithography as originally described by Jeon et al.[3] Briefly, a high-resolution printer was used to generate a mask with a minimum feature size of 30 µm from a CAD file (CAD/Art Services, Poway, CA). A SU-8 2100 photoresist (Microchem, Newton, MA) layer was spin-coated onto a silicon wafer at 3500 rpm for 30 s and exposed to ultraviolet light for 150 s through the mask in a Karl Suss mask aligner. The wafer was then immersed into SU-8 developer, and the unexposed photoresist was dissolved, leaving behind a master mold composed of 100-µm high crosslinked photoresist structures. Poly(dimethylsiloxane) (PDMS) chips were formed by curing prepolymer solution (Sylgard 184, Dow Corning) on silicon masters. Inlet and outlet ports were punched out of the PDMS using a sharpened needle. Polyethylene tubing was inserted into the ports to enable fluid flow into and out of the microchannels.

Measurement of protein surface density. Lyophilized samples of FNZE or QKZE (1 mg each) were dissolved in 0.5 mL of $NaHCO_3$ buffer (100 mM, pH~9, adding SDS until dissolution) and treated with 0.5 mg of the NHS esters of either Cy3 (Amersham, for FNZE) or Alexa 647 (Invitrogen, for QKZE) for 5 h at room temperature. Dialysis was used to remove unconjugated dye. Protein solutions (2 µL each) at different concentrations (0.2, 0.1 and 0.05 µM of FNZE; 0.4, 0.2 and 0.1 µM of QKZE) were spotted onto ZRELF-coated glass slides and dried in air overnight. The fluorescence intensity and area of each spot were measured by a GenePix 4200A chip reader. The

surface density at each spot was calculated and plotted against fluorescence intensity.

Generation of immobilized protein gradients. The PDMS chip and glass substrate (with the ZRELF-coated region covered [4]) were activated with oxygen plasma (200 millitorr, 35 s, 80 W, Anatech) and coupled immediately to form an irreversible seal. To remove trapped bubbles and block non-specific protein adsorption, microfluidic channels were flushed with 2% BSA solution for 30 min using a PHD 2000 syringe pump (Harvard Apparatus). Protein solutions in 2% BSA were injected into the mixer inlets at a rate of 0.5 µL/min for 1 h to generate immobilized protein gradients. The assembly was then soaked in water and the PDMS directly above the exit channel was cut away with a razor blade, leaving the protein gradient positioned within a PDMS well on the glass slide. The sample was sonicated in PBS for 30 min and blown dry, and the gradient profile was scanned by a GenePix 4200A chip reader. For substrates used for cell studies, PBS was left in the PDMS well to prevent dehydration of the gradient surface.

Cell culture. Human umbilical vein endothelial cells (HUVECs, Clonetics) were maintained in a 37℃, 5% CO_2, humidified environmental chamber. The cells were grown in endothelial cell basal medium (EBM-2, Clonetics) supplemented with the supplied Bulletkit, which was replaced every 2 days. Near-confluent HUVEC cultures were passaged nonenzymatically by treatment with 0.61 mM EDTA (Gibco). Passages 3–6 were used.

Cell studies. The gradient region of the glass slide, including the PDMS well, was separated from the rest of the slide by using a diamond pen and placed in a six-well tissue culture plate. The plate was sterilized by ultraviolet light exposure for 5 min in a laminar flow hood. HUVECs re-suspended in 5 mL serum-free EBM-2 containing 2% BSA were added to the samples at a density of 10 000 cells /cm^2. After 2 h, the plates were gently washed twice with EBM-2 containing 2% BSA, and imaged using a 10x phase contrast objective on a Nikon Eclipse TE 300 inverted microscope. Images were captured on a Sony CCD color video camera (model DXC-151A) equipped with Metamorph software. Fifteen images taken randomly from 3 gradient substrates were used to quantify cell attachment.

References

[1] K. C. Zhang, M. R. Diehl, D. A. Tirrell, *J. Am. Chem. Soc.* **2005**, *127*, 10136.

[2] K. Kirshenbaum, I. S. Carrico, D. A. Tirrell, *ChemBioChem* **2002**, *3*, 235.

[3] N. L. Jeon, S. K. W. Dertinger, D. T. Chiu, I. S. Choi, A. D. Stroock, G. M. Whitesides, *Langmuir* **2000**, *16*, 8311.

[4] X. Y. Jiang, Q. B. Xu, S. K. W. Dertinger, A. D. Stroock, T. M. Fu, G. M. Whitesides, *Anal. Chem.* **2005**, *77*, 2338, see Figure 7.