Supplementary Material (ESI) for Lab on a Chip

Microfluidic fabrication of addressable tethered lipid bilayer arrays and optimization using SPR with silane-derivatized nanoglassy substrates

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Electronic Supporting Information

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EXPERIMENTAL

Materials

L-α-phosphatidylcholine (PC), L-α-phosphatidylethanolamine (PE), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt) (biot-PE) were purchased from Avanti Polar Lipids (Alabaster, AL). Cardiolipin sodium salt from bovine heart was from Sigma (St. Louis, MO) and 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC) was from Molecular Probes (Eugene, OR). All lipids were made into stock solutions in chloroform and stored in the freezer, except NBD-PC, which was stored in ethanol. Biotinylated bovine serum albumin (biot-BSA), streptavidin, neutravidin and avidin were from Pierce Biotechnology, Inc. (Rockford, IL). Poly(ethylene glycol) (average molecular weight 8000 g mol^{-1}) (PEG-8000) and fluorescein isothiocyanate labeled Cholera Toxin B subunit (CT-FITC) were from Sigma (St. Louis, MO). The monosialoganglioside receptor (GM1) was from Matreya (Pleasant Gap, PA). Triethyloxysilane aldehyde for use on nanoglassy SPR substrates was obtained from United Chemical Technologies, Inc (Bristol, PA). All proteins and vesicle solutions were diluted with phosphate buffered saline (50mM PBS; 150mM NaCl; pH 7.4), the same buffer used for all SPR, microchip and flow injection experiments. Dow Corning Sylgard 184 silicone elastomer base and curing agent were obtained from a local supplier. All organic solvents were HPLC grade and used as received.

PDMS extraction

PDMS chips were prepared by mixing ten parts PDMS base with one part curing agent, degassed under vacuum, poured onto an aluminum master mold previously described[1] and cured for 1 h at 70ºC. Extraction of PDMS was based on methods described by Whitesides and co-workers.[2] PDMS chips were first immersed in a highly swelling solvent (diisopropylamine) for 1 day at 25ºC with intermittent mixing, then subsequently de-swelled by immersing them in decreasingly soluble solvents that were miscible with diisopropylamine. These included toluene, ethyl acetate, and acetone.

Formation of small unilamellar vesicles

Vesicle solutions for fluorescent characterization were prepared from stock solutions in chloroform. Typically, 43% PC, 35% PE, 18% cardiolipin, 1% biot-PE and 3% NBD-PC were mixed together, gently purged with nitrogen to form a dry lipid film in small amber vials, then rehydrated with PBS to a lipid concentration of 1 mg/mL and vigorously vortexed. The suspended lipids were probe tip sonicated with a sonifier for 20 min. Any titanium particles released from the probe tip were removed from the multilamellar vesicle solution by centrifugation, and the supernatant extruded 19 times with a polycarbonate membrane of pore size 100nm to create small unilamellar vesicles (SUVs). This solution was incubated at 4ºC for 1 h.

Cross patterning

Vantage aldehyde slides were purchased from CEL Associates, Inc. (Pearland, TX), onto which an extracted PDMS microchip was carefully assembled and clamped down. Biot-BSA (0.5mg/mL) was then injected through 3 channels (200µm x 200µm x 2cm) and allowed to incubate for 30 min. After subsequent rinsing with PBS through a syringe pump (KD Scientific, Holliston, MA) with low-pressure sample injectors (Upchurch), the buffer was pulled out of the channels and the extracted chip was lifted off of the slide. The flow rate was fixed at 1 mL/h for all steps throughout the experiments. A regular (non-extracted) PDMS chip was then aligned 90º to that of the biot-BSA on the slide, allowing the rest of the experiment and quantitative fluorescent detection to be accomplished with this chip still intact.

Protein sublayer

Once the non-extracted PDMS chip was carefully assembled, the surface aldehyde groups within the 3 channels were passivated with a 1 mg/mL solution of BSA for 1 h. After rinsing 10 min with PBS, neutravidin, streptavidin or avidin was co-injected with BSA at a concentration of 0.20 mg per 1 mL of 1% (w/v in PBS) BSA solution. The streptavidin was allowed to incubate for 1 h in order to allow sufficient binding to the small biot-BSA zones, then rinsed for 10 min with PBS.

Tethered membrane formation

The biotinylated SUVs were injected into the microchannels, incubated for 1 h, and subsequently rinsed. PEG-triggered fusion of the SUVs was adapted from methods described by Bourdillon, *et al*.[3] Fusion of the immobilized vesicles was triggered by injecting a concentrated solution of PEG-8000 (30%w/v in PBS) through the channels. After each channel had been in contact with this solution for 8 min, the channels were rinsed for 15 min.

Fluorescence imaging and FRAP

Fluorescence recovery after photobleaching (FRAP) experiments were used to determine whether PEG-8000 fusion had successfully created a fluid bilayer. A Meridian Insight confocal laser scanning microscope (CLSM) with 488nm argon laser excitation, SPOT Pursuit CCD, and fluorescein emission filter was used in conjunction with a 40x/0.75na Achroplan dipping objective. The PDMS chip clamped to the aldehyde derivatized glass slide was left intact and immersed in deionized water. The dipping objective was focused onto the surface of the aldehyde slide. Quantitation of the lateral diffusion was achieved by stopping the scanning mode of the CLSM, bleaching a perpendicular line through a 95μm aperture for 0.5 s, and monitoring the fluorescence recovery with time.

In order to image the entire 3x3 fluorescent array, a Leica stereo dissection microscope with a 100W mercury arc lamp was used in combination with a GFP3 filter set. For quantitative CT binding experiments, an Amersham Typhoon 9410 scanner with 488-nm excitation was used, collecting emission using a 520 BP 40 filter.

Surface plasmon resonance spectrometry

For SPR measurements, a Biosuplar instrument (nanoSPR, USA) was employed. Nanoglassy substrates fabricated as previously described[4] were used to simulate a glass surface. The substrates were reacted with an ethanol solution containing 2% triethyloxysilane aldehyde, 4% water and 0.1% acetic acid for 1 h. Following the reaction, the substrates were rinsed with absolute ethanol and heated at 120°C for 10 min in a nitrogen atmosphere.[5]

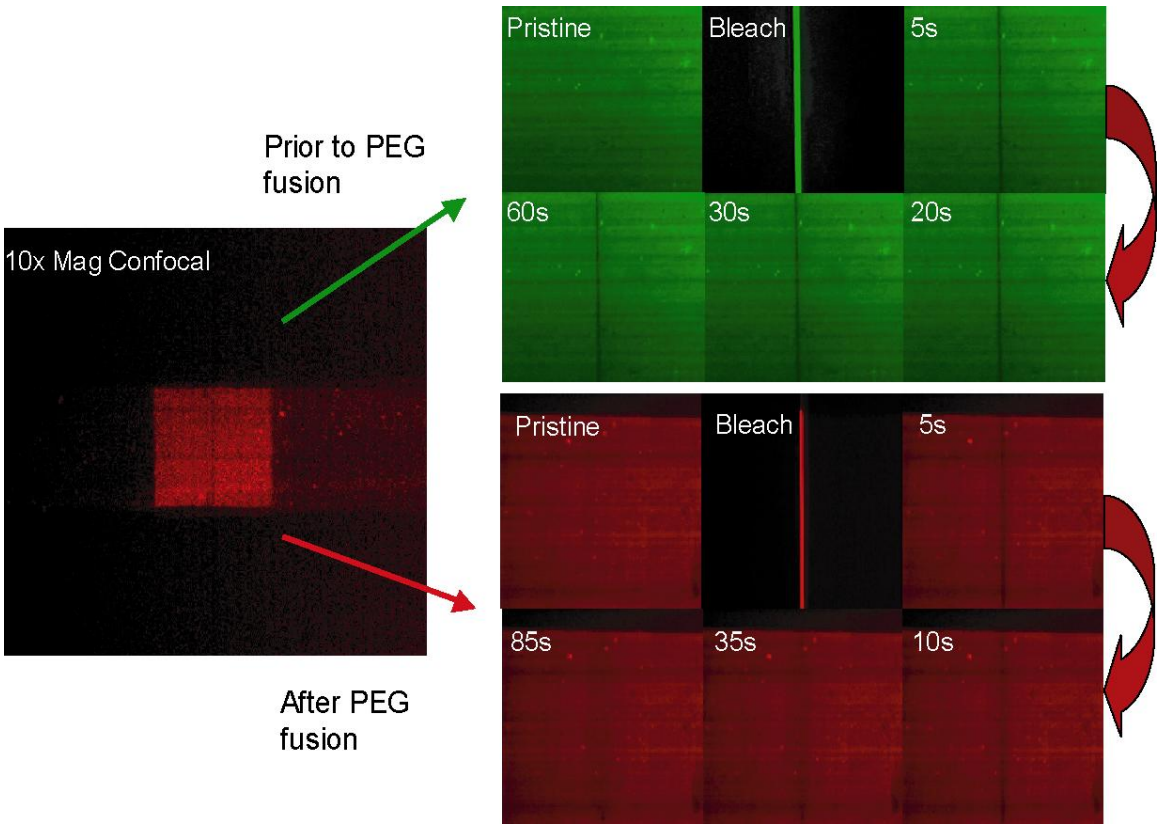

Figure 1S FRAP experiments demonstrating the triggered fusion using PEG-8000. The top series (green) was obtained prior to injecting PEG-8000. The bottom series (red) represents after PEG-8000 injection. Recovery was nearly complete in less than 2 min.

References

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