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ONLINE METHODS

Fabrication of micro-well array

Micro-well arrays were fabricated according to the method reported previously (10). In brief, a hydrophobic polymer of carbon-fluorine (CYTOP, Asahi-glass, Japan) was spin-coated on a clean coverglass (Matsunami, Japan) at 2000 rpm for 30 s and then baked for 1 h at 180°C. This process was repeated 5 times to reach a thickness of 5 μm. Then, photolithography was carried out with a positive photoresist (AZP4903, AZ Electronic Materials, Japan) to pattern the mask structure on the CYTOP layer. A resist-patterned substrate was dry-etched with O_2 plasma by a reactive ion etching system (RIE-10NR, Samco, Japan). The substrate was then cleaned and rinsed with acetone and ethanol to remove the photoresist layer.

PC12 Cell preparation

PC12 was purchased from the Riken cell bank (Ibaraki, Japan) and maintained in DMEM/F12 (Gibco) supplemented with 10% horse serum (HS), 5% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin. To differentiate PC12, cells were cultured for 4 days in the presence of 50 ng/ml nerve growth factor (NGF). For experiments, cells were harvested with 0.25% trypsin, washed twice with HEPES buffer comprising 25 mM HEPES, 140 mM NaCl, 2.7 mM KCl, 0.49 mM $MgCl_2$, 1.8 mM $CaCl_2$, 12 mM $NaHCO_3$, 0.37 mM KH_2PO_4 and 5.6 mM glucose at 1.0×10^5 cells/ml. To depolarize the cell, NaCl concentration was decreased to 80 mM and 60 mM KCl was added. Then, 500 μl of cell solution was placed onto the micro-well array, which was attached to a 35 mm culture dish, and left for 5 min to allow cells to settle at the bottom of the dish. Finally, FC-40 (Sigma-Aldrich) was gently pipetted onto the micro-well, and displaced excess buffer was discarded. After 1 hour incubation, buffer surrounding the cell was collected and applied to MS.

T- and B-cell preparation

Spleen and lymph node cell suspensions were prepared from 5-8 week old BALB/c mice and stained for CD90.2 for T-cells or CD8a and CD45R for B-cells. Individual T- and B-cells were separated using a MACS magnet cell trap system (Milteny Biotec, Bergisch Gladbach, Germany) according to the manufacture's protocol. Cells were washed and suspended in HEPES buffer comprising 25 mM HEPES, 140 mM NaCl, 2.7 mM KCl, 0.49 mM $MgCl_2$, 1.8 mM $CaCl_2$, 12 mM $NaHCO_3$, 0.37 mM KH_2PO_4 and 5.6 mM glucose at 1.0×10^5 cells/ml. Then, 500 μl of cell solution was placed onto a micro-well array, which was attached to a 35 mm culture dish and left for 5 min to allow cells to settle at the bottom of the dish. Finally, FC-40 (Sigma-Aldrich) were gently pipetted onto the micro-well, and displaced excess buffer was discarded. All chemicals used were reagent grade.

Single cell mass spectrometry

MS analysis was performed on an apparatus (LTQ Orbitrap Velos Pro, Thermo Fisher Scientific Inc.) equipped with a nano-electrospray ion source. The spray voltage was set at 1000 V with a distance of 1.5 mm between the top of the needle and the mass spectrometer inlet. The selected range of measurement was from m/z 100 to 300. A micro-well

array seeded with cells was mounted onto an inverted microscope (IX-81, Olympus, Japan), and a micro-well with a single cell was selected for the sample collection. A metal-coated glass capillary nanospray tip (Humanix, Hiroshima, Japan) with tip diameter of 2~3 μm attached to a micromanipulator (MHW-103, Narishige, Tokyo, Japan) was used to sample the extracellular fluid using a tube-connected piston syringe. An ionization solvent (1 μl 80% methanol containing 0.1% formic acid) was added to the collected sample, and the nanospray tip was set on a nano-electrospray ionization (ESI) ion source attachment.

Data analysis

Peaks corresponding to the ionization solution were removed before the analysis. Peaks detected in micro-wells with cells were compared with those from micro-wells without cells (blank), and peaks that were significantly different from blank ($p < 0.05$) were analyzed. Principal component analysis and discriminant analysis was performed using MarkerView software (AB Sciex, Framingham, MA).