A hyphenated optical trap capillary electrophoresis system for single-cell chemical analysis

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Supplemental Information

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2. Optimizing the coverslip coatings and additives.
3. Figure S1. Interfacing the optical trap and the MC-CE-LINF instrument.

1. Preparation of the inlet of capillary electrophoresis column

The 85–120 cm in long CE columns were made from 50 μm inner diameter, 360 μm outer diameter fused silica capillaries (Polymicro Technologies, Phoenix, AZ). Hydrofluoric acid (HF) etching of the capillary inlet and outlet was performed to reduce their outer diameters and create sharply tapered tips with a 40° angle.[1] The ends were scored and snapped to provide a relatively even surface for etching. Approximately 1 cm of the capillary's polyimide coating was burned off of each end and the tips cleaned with methanol. A container was filled to a 5 mm depth with 48% HF and covered with isooctane to prevent HF fumes from rising. The capillary tip was pushed through a FEP sleeve (Upchurch Scientific, Oak Harbor, WA) held tightly in a customized Teflon holder, which maintained the tip position during etching, until the tip touched the bottom of the container. The capillary had isooctane continuously pumped through the non-submersed end via a syringe to prevent the inner walls of the submerged end from being etched. After two hours, the etched tip was rinsed with $Na_2B_4O_7 \cdot 10H_2O$ (Borax, Henkel Corp., Billerica, MA) and water and the process was repeated for the other end.

2. Optimizing the coverslip coatings and additives

Materials and methods

In order to prevent the cells from sticking to the coverslips, a number of different coatings were tested with the results reported here. Tests were performed by pipetting 2.5 to 5 μL of cell suspension (pinealocytes, olfactory bulb cells, or cerebellum cells) in mGBSS onto the treated coverslip or pipetting the additive solution into an aliquot of the cell suspension and transferring that onto a coverslip. The optical trap was operated at 1.3 W to 1.5 W initial power, and the capillary was controlled by the micromanipulator. Video was recorded for all tests using a monochrome CMOS camera.

Bovine serum albumin (BSA): (1) Coverslips were sonicated with isopropyl alcohol for 10 min, rinsed with ultrapure deionized (DI) water, and dried. BSA was dissolved in phosphate buffered saline (BioWhittaker, Lonza, Walkersville, MD) (0.01 g/mL). Coverslips were soaked in the BSA solution for 10 min. Excess BSA was rinsed off with phosphate buffered saline and the coverslip dried using compressed air. (2) Coverslips were sonicated in 25% w/v NaOH (200 mL) + 95% ethanol (600 mL) for 10 min, rinsed by dipping into five individual containers of ultrapure DI water, and dried in a 45°C oven. BSA (10 mg/mL) was dissolved in 80 mM PIPES

(piperazine-N,N'-bis(2-ethanesulfonic acid), 6.05 g, dissolved in 250 mL ultrapure DI water, titrated to pH 6.8 with 10 M NaOH) and filtered by a 0.22 μm syringe filter (Nalgene, Rochester, NY). The coverslips were coated with the BSA solution and immediately rinsed by dipping into five individual containers of ultrapure DI water.

Parafilm M: Coverslips were washed with Alconox (Powdered Precision Cleaner, Alconox Inc., White Plains, NY), rinsed with ultrapure DI water and methanol, and dried. Parafilm M (Pechiney Plastic Packaging Inc., Chicago, IL) was stretched over the surface of the coverslip.

Plastic coverslips: Plastic coverslips were used without cleaning or treatment.

Glass Free: Coverslips were washed with Alconox, rinsed with ultrapure DI water and methanol, and dried. Next the coverslips were submerged in Glass Free (National Diagnostics, Atlanta, GA) for 5 min in a ventilation hood, then rinsed with toluene followed by methanol. The coverslips were gently buffed with a paper towel until dry and stored in a plastic bag, interleaved with paper until use.

Poly(2-hydroxyethyl methacrylate) (pHEMA): Coverslips were washed with Alconox, rinsed with ultrapure DI water and methanol, and dried. pHEMA solution (2.5 % w/v) was prepared by dissolving 6 g of pHEMA in 50 mL of 95% ethanol, stirred overnight at 37°C, and filtered by a 0.22 μm syringe filter (Nalgene) before use. pHEMA solution was pipetted onto coverslips and allowed to evaporate until dry.

Sigmacote: Coverslips were washed with Alconox, rinsed with ultrapure DI water and methanol, and dried. Sigmacote (Sigma-Aldrich, St. Louis, MO) was applied to the coverslips and allowed to dry. Dried coverslips were rinsed with ultrapure DI water before use.

Ethylenediaminetetraacetic acid (EDTA): EDTA solution (5 mM) was prepared by dissolving 0.018 g EDTA in 10 mL mGBSS. The final concentration of EDTA in the cell suspensions was 2.5 mM.

Nanodiamonds: Nanodiamond (ND98, Dynalene Inc., Whitehall, PA) suspensions were prepared by adding 5% w/v of nanodiamonds to 1 mL of mGBSS and sonicating for 60 min. The final concentration of nanodiamonds in cell suspensions was 2.5% w/v.

Ethylene glycol: Ethylene glycol solution (~10% v/v) was prepared by adding 1 mL of ethylene glycol to 9.5 mL of mGBSS. The final concentration of ethylene glycol in cell suspensions was 5% v/v.

Results and discussion for coverslip coatings and additives

BSA was first tested as a coverslip coating, using two different preparation methods. BSA is commonly used as an all-purpose 'blocking' agent in order to occupy the majority of sites that other proteins and molecules would bind to on a surface, preventing cellular adhesion. Pinealocyte adhesion was tested, and BSA-coated coverslips were found to be ineffective. Cells could be loosened more easily using the capillary inlet on BSA-coated coverslips, but pinealocytes still adhered within a few minutes and trapping was unable to be performed.

Diluting the pinealocyte suspension by a factor of two with mGBSS did not lessen the adhesion, reinforcing the fact that it was due to the covalent interaction of the cells' membrane proteins with the glass surface. Plastic coverslips were used to see if the covalent interactions would be lessened; this was not the case. Parafilm M was also tested, and it was effective in eliminating pinealocyte adhesion; however, the film interfered with the use of the optical trap.

Since pinealocytes were not responding to any of the above methods to reduce adhesion, cells from two other brain regions were also tested to determine if the degree of adhesion observed with pineal cells was unique or common to brain cells, since previous experiments with blood cells did not display this behavior (data not shown). Cerebellum cells (*e.g.*, Purjinke cells, glia, granular cells) and olfactory bulb cells (*e.g.*, mitral cells, periglomerular cells, granular cells) were used for comparison, and cells from all three brain regions were exposed to a variety of coatings and additives. Olfactory bulb and cerebellum cells were first monitored on untreated glass coverslips; both exhibited little to no response to the optical trap or the capillary inlet within minutes of exposure to the surface. Cerebellum cells were slightly more responsive to capillary in-flow and out-flow after adhesion than olfactory bulb cells and pinealocytes.

Silicon-based solutions were tested next. Glass Free, a silanizing agent, is typically used to coat glass casting plates for easy release of polyacrylamide gels. Sigmacote is a silicone solution in heptane that readily forms a covalent, microscopically thin film on glass and is used to prevent clotting of blood plasma on surfaces and is water repellent. Glass Free was mildly effective in reducing the adhesion of olfactory bulb cells and pinealocytes but not cerebellum cells; trapping and manipulation was still challenging. Sigmacote noticeably reduced adhesion for olfactory bulb and cerebellum cells and both of these samples could be trapped and manipulated for several minutes. Pinealocyte adhesion appeared reduced, but not as much as for the other brain regions' cells and pinealocytes demonstrated little response to the optical trap.

pHEMA is a polymer that is used in soft contact lens, forms a hydrogel in water, and has been shown to reduce adhesion of brain cells on glass surfaces.[2,3] The response to pHEMA-coated coverslips was positive; all three brain regions' cells displayed reduced adhesion and were able to be trapped and manipulated for several minutes. Pinealocytes were the most affected, demonstrating greater mobility compared to olfactory bulb and cerebellum cells.

Several different additives were tested, which included ETDA, a nanodiamond suspension, and ethylene glycol. These additives were tested on untreated coverslips and pHEMA-coated coverslips. EDTA is a chelating agent and is used in tissue culture for, among other actions, detaching adherent cells for passaging. EDTA had no effect on pinealocyte adhesion but it was very effective in eliminating adhesion of cerebellum and olfactory bulb cells to uncoated coverslips. Olfactory bulb cells in particular were easy to trap and manipulate. The nanodiamond suspension consisted of 5 nm nanodiamonds (potentially carbon nanotubes) in powered form, and according to the manufacturer's website its uses range from drug delivery to separations to biologically-resistant coatings. Pinealocyte adhesion was slightly improved and some response to the trap was observed on uncoated coverslips; reduced adhesion was observed for the other cell types and trapping and manipulation could be performed. On pHEMA-coated coverslips, the nanodiamond suspension reduced adhesion for all cell types, and, interestingly, clustering was observed for pinealocytes. Trapping and manipulation could be performed on all cells.

Ethylene glycol can be used as an alternative to formaldehyde for preserving samples and may reduce interactions between cells and surfaces. It had very little effect on pinealocytes, and no effect on cerebellum or olfactory bulb cells on untreated coverslips; however on pHEMA-coated coverslips, ethylene glycol reduced adhesion noticeably for all cells and made trapping and manipulation possible. Part of this response (and the responses observed in previous additive experiments) may be due to reduced viscosity, as it is easier to trap objects in less viscous solutions, although this was not observed for diluted pinealocytes on untreated coverslips. To further explore this phenomenon, suspensions of olfactory bulb and cerebellum cells were 2-fold diluted with mGBSS and mGBSS + 5 mM EDTA and observed on uncoated coverslips and pHEMA-coated coverslips. Cells that were diluted with mGBSS were easier to trap and manipulate on pHEMA-coated coverslips compared with undiluted suspensions, and mGBSS + EDTA-diluted suspensions on pHEMA-coated coverslips were actually a little more difficult to

trap but still improved compared with undiluted suspensions. Diluted suspensions on uncoated coverslips were easier to trap and manipulate compared with undiluted suspensions on uncoated coverslips as well. This discrepancy in behavior between pinealocytes and other brain cells is only partially explained by viscosity, since pinealocyte suspensions exhibit low viscosity compared with olfactory bulb and cerebellum cell suspensions but still adhere strongly to untreated and treated coverslips, regardless of dilution.

Based on the results of these tests, pHEMA-coated coverslips were subsequently used for this study.

References

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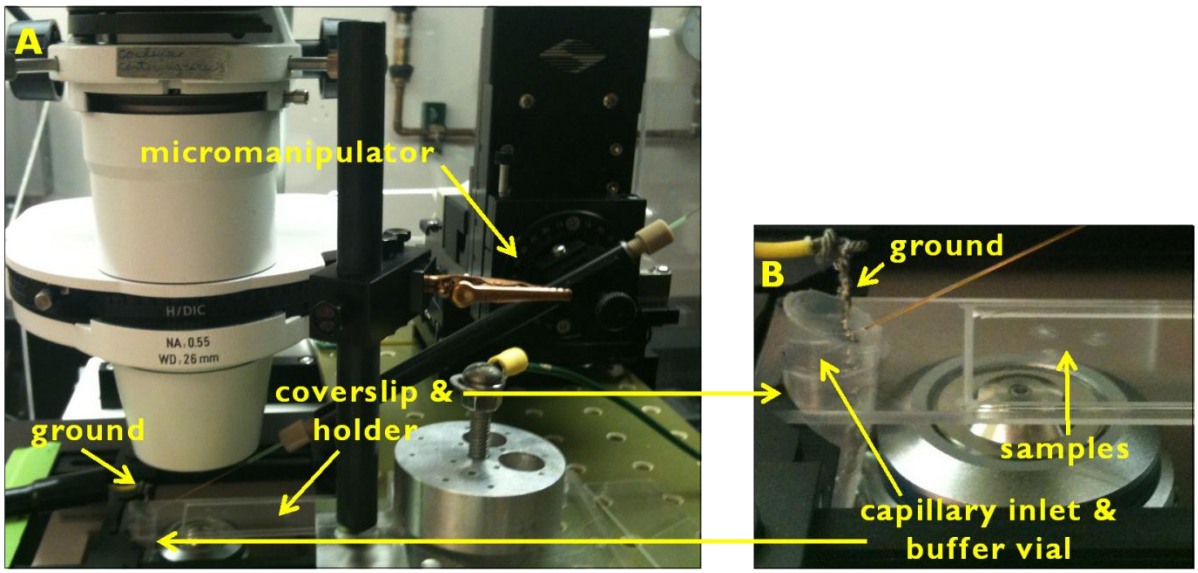

Figure 1S. Interfacing the optical trap and the MC-CE-LINF instrument. (A) An overview of the micromanipulator holding the capillary in position in the buffer vial. (B) A close up view of the sample holder.