

Lab on a Chip

Supplementary Information – Fabrication and Experimental Procedure

Title: Microfluidic system for toxicity testing with integrated electroosmotic pumps, concentration gradient generator and fish cell line (RTgill-W1) – towards water toxicity testing

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Experimental Information

Materials

The poly(dimethylsiloxane) (PDMS) prepolymer and curing agent were purchased from Dow Corning (Sylgard 184). SU-8 negative photoresist and developer were purchased from MicroChem (Newton, Ma). The Bind-Silane solution consists of 0.8%(v,v) 3-(trimethoxysilyl)propyl methacrylate, 0.8%(v,v) acetic acid, pH 3.5 in ultra pure water. The gel salt bridge solution consists of 18%(w,v) acrylamide, 3%(w,v)N-methylenebisacrylamide and 3%(w,v) 2-dimethoxy-2-phenylacetophenone in isopropanol. Chemicals for the Bind-Silane, gel and sodium dodecyl sulfate (SDS) solutions were obtained from Sigma (St. Louis, MO). Fluorescein dye (F-1300) and the Live/Dead assay (L-3224) were obtained from Invitrogen. All solutions were prepared with filtered (0.2 μ m) ultra pure water (MilliQ, Millipore). For cell attachment a solution of fibronectin (F-4759, Sigma) in Delbecco's PBS was prepared. L15ex was prepared from the following recipe:136mM NaCl, 5mM KCl, 1.6mM MgSO₄, 2.1mM MgCl₂, 1.26mM CaCl₂, 1.3mM Na₂HPO₄, 0.36mM KH₂PO₄, 5mM Galactose, 6.7mM Pyruvate in filtered sterile ultra pure water.

Fabrication of Electroosmotic Pumps and Toxicity Chip

Microfluidic devices were fabricated from PDMS using conventional soft-lithography techniques.^{1, 2} Briefly, masks for fabricating SU-8 masters were designed in AutoCAD and printed in high resolution (20k dpi) on Mylar films (CAD/Art Services). The EO pumps and toxicity chip designs were patterned in SU-8 photoresist on separate 4" silicon wafers. Each master includes three replicas of the main design. The EO pump design contains multi-level structures of different height. The EO channels were fabricated using SU-8 2001 (1.9 μ m thick); afterwards, SU-8 2025 was spun on top and the gel regions were processed (95.4 μ m thick). The toxicity chip contains only one level of features that were fabricated using SU-8 2025 (89.7 μ m thick). An optical profiler (Wyko NT1100, Veeco) was used to measure the dimensions of the relief features.

PDMS was mixed in a ratio of 10:1 base to curing agent and degassed for 30 min in a vacuum chamber. The liquid PDMS was poured onto each master and cured at 95°C for 12hr. Molds were then cut out, trimmed and fluid access holes were punched. Microscope slides were ultrasonic cleaned in strong detergent for 10min, followed by 10min in ultra pure water, washed with ethanol, blow dried and baked at 200°C for 10min. The slide and PDMS mold were then air plasma treated (PDC-001, Harrick Plasma) for 60s at 29.6W, then the two pieces were bonded.

Toxicity Chip Fabrication

After bonding, the gradient generator design was checked under a microscope to verify that the device was defect free. This step is important since the gradient profile depends strongly on the geometric design of the microfluidic channels. Then the chip was filled with ultra pure water, sealed with Kapton tape and placed under a UV sterilization lamp for 6hr. Completed chips were stored in sterile bags until they were needed for toxicity experiments.

EO Pump Gel Salt Bridge Fabrication

Gel salt bridges were fabricated using a photolithography process as depicted in Fig. S1. Immediately after bonding, the EO pumps were filled with a freshly made Bind-Silane solution for 2hr. Plasma treatment is a precondition for the process to work effectively, since it exposes silanol groups, creating reaction sites for the Bind-Silane molecules. Bind-Silane is a bifunctional molecule that participates in the polyacrylamide (PAA) reaction and creates a bond between the PAA gel and PDMS/glass, preventing leakage and increasing mechanical stability.^{3,4}

During the Bind-Silane treatment, the gel regions on the EO pumps were masked off with tape for the ensuing photopolymerization. The gel solution was introduced into the chip by vacuum and the gel was polymerized by UV light using a microscope with a 20X objective (GX-71, Olympus) and 100W mercury burner with a 50% neutral density filter. The polymerization was monitored *in situ* with a CCD camera (CoolSNAP ES, Photometrics). The total time for processing all three EO pumps was 10min.

Afterwards, each EO pump was flushed by a series of solutions using a syringe pump to control the flow rate (11 Plus Dual Syringe Pump, Harvard Apparatus). The solutions were flushed from the fabrication inlets to the outlets of the EO pumps, so as to reduce the risk of clogging the narrow EO channels. Each pump was flushed first with a 10% (v,v) solution of IPA to remove residual gel monomer, then by a solution of 0.5% (w,v) NaOH to remove the Bind-Silane, and finally by ultra pure water to swell the gel. Each solution was flushed at a flow rate of $20\mu\text{L min}^{-1}$ for 10min.

The fabrication access holes were then plugged with silicone sealant (732 RTV, Dow Corning) and reservoirs were attached. The EO pumps were filled with a 5mM Sodium Borate solution, sealed and left for two days before operating the pumps.

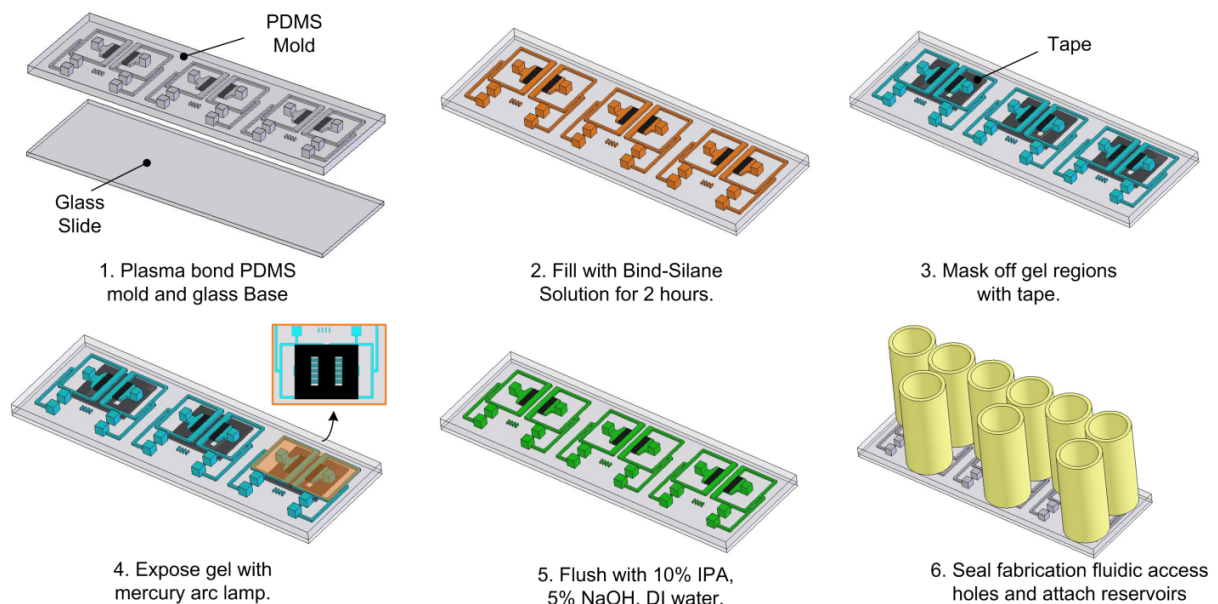


Fig. S1 Fabrication procedure for the EO pumps used in the toxicity studies. PDMS mold is bonded to a cleaned glass slide (1) and filled with Bind-Silane solution for 2hr (2). The chip is filled with the gel solution and the gel regions are taped off (3). The gels are photopolymerized by UV light on a microscope using a mercury arc lamp and CCD camera to monitor the process (4). The pumps are then flushed with solutions of 10% IPA, 0.5% NaOH and DI water (5). Finally, the fabrication access holes are sealed and reservoirs are attached (6) to complete the chip.

RTgill-W1 Cell Culture

The RTgill-W1 cell line was initiated from primary cultures of rainbow trout gill cells.⁵ Cells were maintained in non-vented flask at room temperature (22°C) with L-15 (Sigma Aldrich) media supplemented with 10% (v,v) fetal bovine serum, 100U mL⁻¹ penicillin and 100µg mL⁻¹ streptomycin. Cells used in these experiments have been passaged over 100 times.

Toxicity Testing Experimental Procedure

The protocol for performing the toxicity experiments is summarized in the flow chart presented in Fig. S2. Prior to performing experiments all items used with the cells were sterilized by autoclave. The cell chamber in the toxicity chip was flushed with a 1µg mL⁻¹ fibronectin solution in Delbecco's PBS for 30min to promote cell attachment. The entire chip was then flushed with L-15 medium for an additional 30min. A cells-in-media suspension was made with an average cell density of 1.5x10⁶ cells mL⁻¹. The cell suspension was loaded into a syringe and injected into the cell chamber. The process was monitored under a phase contrast microscope to visualize cell movement. Once the cell density in the chamber was sufficient, the cells were allowed to attach in a static environment for 1hr, followed by perfusion with media for an additional 2hr.

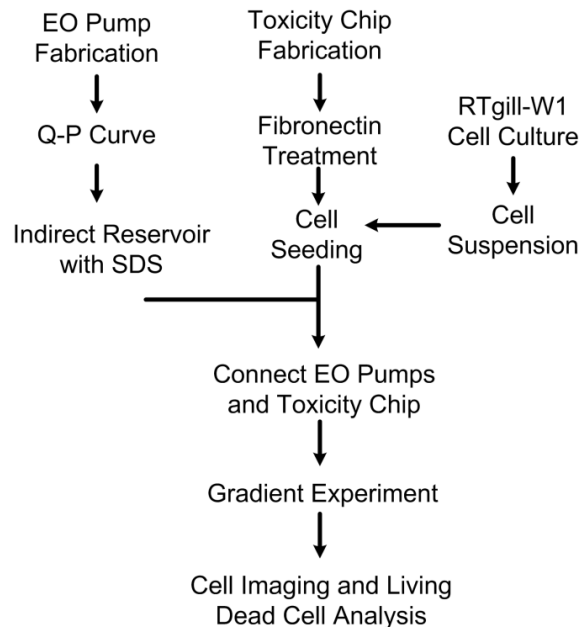


Fig. S2 Flow chart of the experimental procedure for toxicity study with EO pumps.

During this period of time, solutions for the toxicity experiment were made. A serum free base

solution of L15ex was used from which two types of solutions were prepared; one contained normal L15ex and the other L15ex with $50\mu\text{g mL}^{-1}$ of SDS. In addition, components of the Live/Dead cell assay kit were dissolved and vortexed in both solutions. The solutions were then loaded into the indirect pumping reservoirs and attached to EO pumps.

Once the cell attachment period was finished, the EO pumps and toxicity chip were connected. Earlier in the day the Q-P curves for each of the three pumps were obtained and used to determine the required current settings. First, the entire network was flushed with the L15ex using the one EO pump at a flow rate of $2\mu\text{L min}^{-1}$ for 20 min to remove unattached cells and debris. This also allowed for the Live/Dead cell stain to incubate and highlight the cells prior to the experiment. Next the second pump with the SDS was set to $2\mu\text{L min}^{-1}$ and the gradient was allowed to form for 10min. At this point the toxicity experiment began and fluorescent images were recorded at 5min intervals for a period of 1hr using a fluorescence microscope (Eclipse E600, Nikon) with an FITC+Rhodamine filter set and camera (CoolPix E5400, Nikon). The investigation area was located 5mm downstream from the start of the cell chamber. The resulting images were analyzed to determine the fraction of dead cells by overlaying a rectangular mesh (2x3 mm) consisting of 20 rows, which equates to divisions of $2.5\mu\text{g mL}^{-1}$ of SDS.

References

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