A microchip that performs directed capture and chemical activation of surface-modified single cells has been developed. The cell capture system is comprised of digitized gold electrodes microfabricated on a glass substrate within PDMS channels. The cell surface is labeled with thiol functional groups using endogenous RGD receptors, and adhesion to exposed gold pads on the electrodes is directed by applying a driving electric potential. Multiple cell types can thus be sequentially and selectively captured on desired electrodes. Single-cell capture efficiency is optimized by varying the duration of field application. Maximum single-cell capture is attained for the 10-min trial, with 63% (n = 30) of the electrode pad rows having a single cell. In activation studies, single M1WT3 CHO cells loaded with the calcium-sensitive dye fluo-4 AM were captured; exposure to the muscarinic agonist carbachol increased the fluorescence of the original intensity. These results demonstrate the ability to direct the adhesion of selected living single cells on electrodes in a microfluidic device and to analyze their response to chemical stimuli.

The ability to selectively position and interface with single cells from different populations offers many opportunities for studying cell–cell signaling,1 genetic heterogeneity,2 and heterotypic biological systems.3 While significant progress has been made in monitoring bulk electrical, mechanical, chemical, and genetic changes in cell populations, a fundamental understanding of cellular interactions and stochastic effects at the single-cell level has yet to be fully realized.4,5 Measurements from the elements of a heterotypic array of cells reveal unique individual behavior while maintaining their natural context. Ideally a single-cell analysis system would allow for the immobilization, stimulation, and recording (electrical, mechanical, biochemical, or optical) from multiple cell types in a fast, high-throughput, and highly parallel manner. In addition to addressing the opportunities mentioned above, this system may be the precursor to a general bioelectronic interface.

Microfabricated devices provide an excellent platform with which to explore changes and interactions at the single-cell level due to their comparable size scales (1–100 μm), patternable interfaces, and the opportunity for parallel analysis.6 Mechanical cell trapping,7–9 cell sorting,10 optical tweezers,11 dielectrophoresis,12 and electroaddressable array13 microdevices offer the ability to quickly isolate and probe individual cells. Novel surface engineering approaches have been developed for the micropatterning of single-cell attachment islands14,15 and for the co-culture of multiple cell types on a single substrate.16–19 While previous work has demonstrated cell manipulation, patterning, and co-culture of multiple cell types on a single substrate, an integrated microdevice with the ability to position and interface with single cells from different populations has yet to be developed.

Here we demonstrate a novel combination of cell surface modification and electric field-directed adhesion and its use for the rapid capture and chemical activation of living single cells in a microchip. Our approach is to label the cell surface with thiol functional groups using endogenous receptors to the cell adhesion peptide sequence RGD.20,21 The labeled cells are electrophoretically
cally directed to selected gold electrodes, due to their intrinsic negative surface charge. Once captured, the single cells are activated with an agonist to a membrane-bound receptor, and the response is monitored optically with a fluorescent probe. Multiple cell types are sequentially and selectively captured on neighboring electrodes by changing the field direction. Such directed capture of multiple viable cell types in a microfluidic device presents a new paradigm for analyzing the activity and interactions of single cells.

**EXPERIMENTAL SECTION**

**Microchip Fabrication.** Gold electrodes are fabricated on cleaned 4-in.-diameter borofloat glass wafers utilizing a liftoff protocol. Bare glass wafers are treated with hexamethyldisilazane for 5 min to promote photoresist adhesion and Shipley 1818 photoresist (PR) is spun at 2500 rpm for 30 s and cured at 120 °C for 90 s. The interdigitated 40-μm-wide electrodes are patterned in a Karl Suss MAS Mask Aligner through a chrome mask. Exposed positive photoresist is removed in 1:1 diluted Microposit Developer. A 20-nm seed layer of chromium and 100-nm gold film are evaporated onto the patterned glass substrate. The gold electrodes are defined by placing the glass substrates in photoresist stripping solution, PRS-3000 (Mallinkrodt Baker, Phillipsburg, NJ), lifting off chrome and gold from unexposed regions.

An oxide layer was deposited and patterned, leaving three 16-μm² single-cell adhesion pads exposed on each gold electrode. A 24-nm layer of SiO₂ is deposited on the wafer surface using a Ransdex Sputtering System. A masking layer of PR is then lithographically patterned on the surface, with the 16μm² windows centered on the 40-μm-wide electrodes. The oxide is etched in 10:1 BHF for 1 min (etch rate ~25 nm/min) for a 4% overetch. Photoresist is stripped and the wafer is cleaned, resulting in the electrode array shown Figure 1 (inset).

Fluidic channels are formed by placing SU-8 molded poly(dimethylsiloxane) (PDMS) over the gold electrode substrate. Standard photolithography techniques are used to create 6-cm-long, 200-μm-wide, and 32-μm-deep channel molds with SU-8 25 (MicroChem, Newton, MA). Channels are formed by pouring PDMS (1:0.1 Sylgard 184 siloxane elastomer base to curing agent (Dow Corning, Midland, MI) over the SU-8 mold and curing for 48 h at 37 °C. Fluidic access ports are created by punching 0.5-mm holes at each end of the SU-8 defined PDMS channel.

The complete cell capture microdevice is formed by bonding the PDMS channels to the patterned glass substrate (Figure 1). The PDMS substrate is removed from the SU-8 mold and cleaned in a UV ozone oven for 8 min to promote glass–PDMS irreversible bonding. The PDMS substrate is aligned to the glass wafer with alignment marks, contacted, and the substrate sandwich is heated at 100 °C for 15 min to promote permanent bonding.

**PDMA Derivitization.** The bonded glass–PDMS microchannel is derivitized with poly(dimethylacrylamide) (PDMA) using a modified Hjerten coating protocol to prevent nonspecific cell adhesion to glass. First, channels are filled with 1 M NaOH for 1 h to clean and deprotonate the glass surface. After NaOH incubation, the channel is evacuated and filled with a 0.6% (v/v) γ-methacryloyloxypropyltrimethoxysilane solution (γ, Sigma, St. Louis, MO) in 3.5 pH H₂O. The bifunctional γ-solution prepares the glass surface for acrylamide polymer nucleation. During γ-solution incubation, 250 μL of dimethylacrylamide is dissolved in 4.75 mL of H₂O and sparged with Ar for 1 h. After Ar sparging, 100 μL of isopropl alcohol (IPA), 20 μL of TEMED, and 25 μL of 10% (v/v) APS are sequentially added to the acrylamide solution to form linear PDMA. The γ-solution is removed from the channel, and PDMA solution incubates in the channel for 1 h. The channel is then rinsed and dried with acetonitrile.

**Cell Culture.** K1 and M1WT3 strains of Chinese hamster ovary (CHO) cells are cultured using standard techniques for cell culture experiments. All cell culture reagents are obtained from Gibco/Invitrogen Corp. (Carlsbad, CA) unless otherwise noted. Wild-type K1 cells (American Type Culture Collection (ATCC), Manassas, VA, CCL-61) and muscarinic receptor transfected M1WT3 cells (ATCC, CRL-1985) are cultured in parallel using identical techniques. A nitrogen-frozen stock is thawed and grown in F-12 media containing 10% (v/v) fetal bovine serum (FBS, HyClone, Logan, UT) and 1% (v/v) penicillin/streptomycin (P/S, Sigma) for 2 days at 37 °C and 5% CO₂ in T-75 cell culture flasks (Corning, Acton, MA). Adherent CHO cells are grown to confluence and detached from the growth plate by adding 2 mL of trypsin/EDTA and incubating for 5 min at 37 °C. The trypsin/EDTA is neutralized by adding 8 mL of F-12 media containing FBS and P/S. The cell suspension is centrifuged for 3 min at 5000 rpm. The supernatant is aspirated, and the cell pellet is resuspended at 1 x 10⁶ cells/mL in media with FBS and P/S for cell capture experiments.

For muscarinic activation experiments, K1 and M1WT3 cells are deprived of serum for 24 h to arrest the cells’ growth cycle.
Cells are grown for 2 days at 37 °C in a T-75 cell culture flask in media containing FBS and P/S. The medium containing serum is aspirated from the flask and replaced with serum-deprived medium. The remaining cell culture steps are as previously described.

**RGD Binding and Thiolation.** Detached CHO cells are functionalized with thiol-containing RGD peptide. The synthetic peptide, CCRRGDWLC (Sigma Genosys), is used for these experiments because the thiol-containing cysteine (C) is at the amino and carboxyl termini. Detached CHO cells are suspended in 5 mL of 50 μM thiol-containing RGD peptide in phosphate-buffered saline (1×PBS, pH 7.4). Cell suspension is placed in a T-25 flask and gently agitated for 1 h at room temperature to ensure RGD-cell binding and resultant cell thiolation (Figure 2). After 1 h thiolation, cell solution is centrifuged at 3500 rpm for 3 min. Supernatant containing RGD-peptide is removed and frozen for reuse. Initial RGD-cell binding optimization experiments were performed with a fluorescein-labeled synthetic peptide, Flc-CCRRGDWLC. Flow cytometry with the fluorescein-labeled synthetic peptide showed that each K1 CHO cell was effectively labeled with approximately ~5 × 10⁶ thiol groups due to the presence of cysteine residues at both termini of the synthetic nine residue peptide (CCRRGDWLC). Thiol functional groups on the peptide bind to exposed 16-mer gold pads and hold the cell in place.

**CellTracker Dye Labeling.** Thiolated CHO cells are suspended in 1×PBS and labeled with fluorogenic, cell permeant, calcium-sensitive fluo-4 AM dye, to show selective labeling of the muscarinic receptor. For the muscarinic activation assay, wild-type K1 and muscarinic receptor transfected M1WT3 cells are separately cultured and labeled with the green fluorescent fluo-4 AM dye. Both cell types are labeled according to the CellTracker dye labeling protocol, except that cell media with 10% FBS and P/S is used during wash and trials instead of 1×PBS. During the 15-min BSA incubation, 6 μM cell-permeable fluo-4 AM dye is added. Excess dye is removed in the wash protocol previously described.

**Muscarinic Activation Assay.** CHO cells are labeled with the cell permeant, calcium-sensitive fluo-4 AM dye, to show selective activation of the muscarinic receptor. For the muscarinic activation assay, wild-type K1 and muscarinic receptor transfected M1WT3 cells are separately cultured and labeled with the green fluorescent fluo-4 AM dye. Both cell types are labeled according to the CellTracker dye labeling protocol, except that cell media with 10% FBS and P/S is used during wash and trials instead of 1×PBS. During the 15-min BSA incubation, 6 μM cell-permeable fluo-4 AM dye is added. Excess dye is removed in the wash protocol previously described.

Sequential capture of M1WT3 and K1 cells is integrated with the muscarinic activation assay using the same cell preparation and dye labeling protocol. M1WT3 cells are captured on the gold pads first to ensure cells remain viable over the course of the experiment. M1WT3 cells labeled with the CellTracker Blue and calcium-sensitive fluo-4 AM dyes are captured on odd-numbered electrodes according to the cell capture protocol. The driving field is reversed, and K1 cells labeled with the CellTracker Green and calcium-sensitive fluo-4 AM dyes are captured on even-numbered electrodes. After both cell types are captured on gold pads, a 100 μM carbachol solution in 1×PBS is added. Carbachol activates M1 receptors, present at a density of 800 fmol/mg of membrane protein in M1WT3 cells, causing the release of intracellular calcium from stores. ³² Epifluorescent images are analyzed with Image-Pro software (Media Cybernetics) to determine which relative changes in calcium levels. Cell figures were processed by subtracting the background and adjusting the intensity range with ImageJ software.

**RESULTS AND DISCUSSION**

**Single-Cell Capture.** The cell capture microdevice was tested with both thiolated and native CHO cells, in the presence and absence of the driving electric field. For thiolated cells, a 10-min application of the electric field resulted in significant capture of single cells on positive gold electrodes from a suspension of 8.5 × 10⁶ cells/mL (Figure 3A). Total capture increased with capture time; cells continued to be drawn to occupied gold pads leading to cell clumping at 30 min (Figure 3B). Control experiments using thiolated cells but no electric field, or a driving electric field (26) McMillan, R.; Meeks, B.; Bensebaa, F.; Deslandes, Y.; Sheardown, H. J. Biomed. Mater. Res. 2001, 54, 272–283.

without the cell thiolation, resulted in negligible capture (Figure 3C). Statistical analysis confirmed that there was a significant difference between the capture, which combined cell thiolation and electric field and the control trials lacking either component ($\chi^2 > 45$ for $t \geq 5$ min, $P < 0.001$). Similar to previous reports, the focal adhesion point for captured cells occurs at the edge of the gold pad. This suggests that the size of the pad does not limit cell capture and smaller electrodes could be fabricated for increased cell capture density.

Single-cell capture efficiency was optimized by varying the duration of field application. The data were recorded based on capture events on a given electrode, each of which contains three gold binding pads. This reflects the fact that the electrode was the basic independently addressable unit. Figure 4A illustrates how single-cell capture efficiency depends on capture time. Single-cell capture attained maximal efficiency for the 10-min trials for thiolated cells in the electric field, with $63 \pm 9\%$ of rows having a single cell. Increased time led to the formation of cell clumps, which were no longer counted as single-cell capture events. Figure 4B illustrates the result of increased capture time on total capture, where any capture event on an electrode pad was included in the total. This results in a maximum total capture efficiency of $90 \pm 5\%$ for the 60-min incubation.

The total cell capture results exhibited an increase and then a dip before reaching a final maximum. This dip may be due to the manner in which the cells clumped. Capture events for the 10-min trials with thiolated cells and electric field were mostly single cells, which tended to withstand the rinse due to their lower surface area. As the cells clustered loosely, they presented a larger resistance to flow and were more likely to be removed. But as the incubation time increased further, the clumps solidified internally and in their adhesion to the surface, making them more difficult to remove with rinsing.

**RGD Binding and Thiolation.** The use of cell surface thiolation instead of substrate modification presents several advantages. First, it relies on the robust gold–thiol bond instead
of the in situ RGD–integrin interaction. Since the gold–thiol bond is 10-fold stronger than the RGD–integrin bond,\(^\text{28,29}\) this improves capture efficiency. The off-chip incubation in RGD peptide provided \(\sim5 \times 10^6\) thiol groups/cell, which also compares favorably to the estimated 270 000 single strands/cell with DNA-mediated capture reported recently.\(^\text{30}\) Another advantage of cell labeling is that it leaves the electrodes in their native state, which is useful for sensor applications.\(^\text{31}\) In our approach, certain elements of an array of electrodes could be modified for ion selectivity, while adjacent gold electrodes are used for both cell capture and electrical measurements. In the electrode modification approach, the thiol-labeled adhesion molecules form a monolayer on the surface, potentially reducing its recording sensitivity and altering ion selectivity. The cell modification approach thus provides superior adhesion with electrical measurement flexibility.

**Sequential Capture.** Reversing the direction of the electric field after cell capture caused the directed adhesion of cells on the opposite electrodes while leaving the previously captured cells in place. To illustrate this selectivity, the microdevice was used to capture two different populations of CHO cells on electrodes separated by 100 \(\mu\)m (Figure 5). Single CHO cells labeled with the cytosolic dye CellTracker Blue were first captured on the odd-numbered electrodes by setting them at ground potential with the subsequent capture steps. (B) The thiolated K1 cells labeled with CellTracker Blue are introduced through the opposite fluidic port and are captured on the odd-numbered electrode by reversing the 50 V/cm potential. (C) Following incubation with 6 \(\mu\)M cell-permeable calcium-sensitive fluo-4 AM dye, a solution of 100 \(\mu\)M carbachol in 1× PBS is introduced in the channel to selectively activate the muscarinic receptor on the M1WT3 cells. Carbachol binds to the muscarinic receptor, and the subsequent release of intracellular calcium results in an increase of green fluorescence in the M1WT3 cells, while the K1 cells exhibit only faint fluorescence. (D) The blue and green fluorescent images are overlayed, demonstrating the directed capture and selective activation of the two cell types. Bar, 40 \(\mu\)m.

![Figure 5](image_url)

**Figure 5.** Sequential directed capture of two populations of CHO cells. (A) The first population of thiolated K1 cells, labeled with CellTracker Blue, is captured by applying a 50 V/cm potential to the even-numbered electrodes for 10 min. (B) A second population of thiolated K1 cells, labeled with CellTracker Green, is introduced into the channel through the opposite fluidic port and field mediated binding occurs selectively at the odd-numbered electrodes. Bar, 40 \(\mu\)m.

channel, the potential was reversed. The blue fluorescent cells remained bound, while the green fluorescent cells were driven to the even-numbered electrodes by the field (Figure 5B). The final result was the patterning of single cells from two cell populations on alternating electrodes of an interdigitated array.

This technique possesses the important advantage of pattern control and scalability. While it was demonstrated here for only two cell types, our approach could be used to pattern three or more groups of single cells on adjacent electrodes in a sequential manner. This suggests a new approach for parallel drug screening, in which many different cell types are monitored simultaneously in a microfluidic device.\(^\text{32}\) Alternatively, cells could be positioned adjacent to one another to study heterotypic intercellular communication at the single-cell level.

**Single-Cell Capture and Activation.** A cell stimulation assay was integrated with sequential capture to demonstrate that the single CHO cells remained viable and active. M1WT3 cells were loaded with the intracellular calcium indicator fluo-4 AM and captured on the even-numbered electrodes (Figure 6A). K1 cells were loaded with both fluo-4 AM and CellTracker Blue and then captured in the same channel on the odd electrodes (Figure 6B).

![Figure 6](image_url)

**Figure 6.** Single-cell capture and selective activation. (A) Thiolated M1WT3 cells containing the muscarinic receptor and labeled with CellTracker Green are first captured on the even-numbered electrodes. Fluorescent cells not captured on gold pads are washed off in subsequent capture steps. (B) The thiolated K1 cells labeled with CellTracker Blue are introduced through the opposite fluidic port and are captured on the odd-numbered electrode by reversing the 50 V/cm potential. (C) Following incubation with 6 \(\mu\)M cell-permeable calcium-sensitive fluo-4 AM dye, a solution of 100 \(\mu\)M carbachol in 1× PBS is introduced in the channel to selectively activate the muscarinic receptor on the M1WT3 cells. Carbachol binds to the muscarinic receptor, and the subsequent release of intracellular calcium results in an increase of green fluorescence in the M1WT3 cells, while the K1 cells exhibit only faint fluorescence. (D) The blue and green fluorescent images are overlayed, demonstrating the directed capture and selective activation of the two cell types. Bar, 40 \(\mu\)m.

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The channel was then filled with carbachol (100 μM), an acetylcholine analogue, which binds to the M1 muscarinic G-protein coupled receptor, leading to an increase in intracellular calcium. The activation was detected by an increase in fluorescent emission by the fluo-4 AM dye in M1WT3 cells (Figure 6C) while the K1 cells remained unchanged. An overlay of the blue and green images (Figure 6D) indicates the juxtaposition of different cell types and their selective activation.

The box plot in Figure 7 quantitates the on-chip activation of single cells with a statistically valid sample size. Synchronized M1WT3 cells displayed an average sustained fluorescence intensity increase of 110 ± 74% (n = 59) 2 min after initial stimulation. Wild-type K1 cells assayed in the same manner displayed an increase of only 0.3 ± 27% (n = 79) in fluorescent signal due to the absence of the muscarinic receptor (Figure 7A). These data were used to estimate the percentage of captured cells that remained active after capture. The mean of the K1 response plus three standard deviations was chosen as a threshold level for the K1 cells met the threshold (Figure 7A). The difference between the responses of the two types was statistically significant with a χ² value of 88.3 leading to $P < 0.005$ for them being from the same population.

Measurements on single cells display the activity of each cell within the range of the population. The assay performed here demonstrated that the cells were not only viable, but active, and capable of a measurable response to chemical stimuli after sequential capture and sustained electric field exposure. This intracellular calcium assay is a single-cell analogue to a commonly performed bulk screen for receptor agonists and antagonists of pharmacological interest. Our assay demonstrated the same gain order as the conventional fluorometric imaging plate reader format, while providing single-cell resolution.

Because the cells remain competent for response assays, a variety of extensions of this work are possible. While cells were assayed here in the round morphology typical of adherent cells in suspension, the addition of growth media and serum causes the thiolated cells to spread out and grow under culture conditions. The capture technique could be used to direct a single cell to a microelectrode array containing several recording sites, leading to spatial resolution of the cell response or simultaneous recording of multiple analytes. This would provide a bioelectronic interface in which the cell would be in contact with an electrode array capable of chemical or electrical stimulation and recording. Finally, these assays could also be integrated into a microfabricated genetic analyzer for a more complete understanding of both cellular function and genetic makeup at the single-cell level. Once a cell with unique behavior is identified it could be lysed and studied by PCR to identify underlying causes or alterations.

**CONCLUSIONS**

The microdevice developed here presents a simple and effective means for the directed capture and analysis of single cells in a microfluidic chip. The device uses direct labeling of the cells, instead of the more common approach of tailoring the substrate for cell adhesion. The novel combination of the driving electric field and cell thiolation provides adhesion sufficient to withstand subsequent flow used for rinsing or reagent introduction. Additionally, the ability to individually direct the capture of single cells from multiple populations on neighboring electrodes in an integrated microfluidic chip presents advantages over previous work, which demonstrated multicell type patterning. Because the captured cells remain viable, chemical stimulation and optical monitoring reveal cell activity, and additional assays are made possible. This device also provides a novel platform for future single-cell genetic studies as well as the development of a bioelectronic interface for fundamental studies of cell activity.

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