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A microwell device for targeting single cells to electrochemical microelectrodes for high-throughput amperometric detection of quantal exocytosis

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Abstract

Electrochemical microelectrodes are commonly used to detect spikes of amperometric current that correspond to exocytosis of oxidizable transmitter from individual vesicles, i.e., quantal exocytosis. We are developing transparent multi- electrochemical electrode arrays on microchips in order to automate measurement of quantal exocytosis. Here we report development of an improved device to target individual cells to each microelectrode in an array. Efficient targeting (~75%) is achieved using cell-sized micro-well traps fabricated in SU-8 photoresist together with patterning of poly (L-lysine) in register with electrodes to promote cell adhesion. The surface between electrodes is made resistant to cell adhesion using poly (ethylene glycol) in order to facilitate movement of cells to electrode “docking sites”. We demonstrate the activity of the electrodes using the test analyte ferricyanide and perform recordings of quantal exocytosis from bovine adrenal chromaffin cells on the device. Multiple cell recordings on a single device demonstrate the consistency of spike measurements and multiple recordings from the same electrodes demonstrate that the device can be cleaned and reused without degradation of performance. The new device will enable high-throughput studies of quantal exocytosis and may also find application in rapidly screening drugs or toxins for effects on exocytosis.

INTRODUCTION

An important mechanism of cell-to-cell communication is release of transmitter molecules via Ca^{2+}-triggered exocytosis. Exocytosis is a process whereby transmitter-laden intracellular vesicles fuse with the cell membrane and release their contents to the outside of the cell. Since transmitter is released in packets, the process is referred to as quantal exocytosis. Carbon-fiber microelectrodes (CFEs) have been extensively used to study quantal exocytosis of electroactive transmitters such as catecholamines[1–4]. The CFE is placed immediately adjacent to a cell so that each packet of transmitter that is released from the adjacent membrane produces a spike of amperometric current as the transmitter is

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oxidized on the electrode surface. Analyses of amperometric spikes provide unique and detailed information about the amount and time-course of transmitter released during each fusion event. The technique can also resolve the initial flux of transmitter through a nanometer-sized fusion pore[2] (reviewed in [5]) and thus distinguish steps in the fusion reaction. This allows the study of how drugs or protein perturbations affect exocytosis in exquisite detail.

Recently, our group and others have developed microdevices to assay quantal exocytosis[6–17] as an alternative to CFEs in order to enable higher throughput experiments and to provide new capabilities such as simultaneously imaging fluorescence from the same area of the cell surface where exocytosis is assayed electrochemically. An important task in enabling high-throughput experiments with the devices is to automatically target cells to electrodes. Recently our group[15] and others[11, 17] have published reports of using microfluidics to target cells to on-chip electrochemical electrodes. These approaches are attractive in that the assay could potentially be performed in a rapid sequential manner similar to the use of an optical flow cytometer. A drawback of microfluidic positioning is that exocytosis is sensitive to membrane surface tension[18] and occurs spontaneously following mechanical manipulation[13, 17] through a mechanism that may differ from physiological Ca^{2+}-triggering. In addition, it is useful to study exocytosis under conditions where cell adhesion to extracellular matrix proteins is maintained in order to more closely approach physiological conditions and allow combination with other approaches to assay or manipulate individual cells such as TIRF imaging (e.g., [19, 20]) and patch-clamp electrophysiology[3, 8].

There are many reports of patterning cells on microchips using islands of “cytophilic” materials that promote cell attachment separated by “cytophobic” materials that resist cell tethering (see [21, 22] for reviews). Cytophilic materials include extracellular matrix (ECM) proteins or substances that promote ECM adsorption whereas cytophobic materials, such as poly (ethylene glycol), resist protein adsorption. The most common method for patterning cytophobic/cytophilic materials is microcontact printing whereby the material is applied to the substrate using a poly (dimethylsiloxane) stamp (reviewed in [23]). A major challenge for using microcontact printing to pattern cells over electrodes is aligning the flexible stamp with the electrode array with μm precision[24]. Alternatively, photolithographic approaches can be used to pattern cell adhesive domains[25–28], but this approach is challenging because solvents used to strip the photoresist can damage sensitive biomolecules.

We have recently described an approach using nitrogen-doped diamond-like-carbon (DLC:N) as a cytophilic electrode material to promote cell adhesion whereas Teflon AF is used as a cytophobic material that also serves to insulate the conductive film [29]. Whereas we are able to achieve high-efficiency (~66%) targeting of cells to electrodes using this approach, the adhesion of cells to electrodes is weak and could not withstand washing cells with test substances. This motivated us to develop a microwell-based approach to allow more robust trapping of cells over electrodes.

Cell-sized microwells can act as gravity-assisted traps to pattern individual cells on a microchip[30, 31]. Clumps of cells are excluded from the microwells due to size selectivity, allowing on-chip studies of single, isolated cells. In order to facilitate movement of cells to the microwell traps, it is useful to render the surface between the wells cytophobic[31]. In this work we describe development and testing a novel device that uses a combination of microwells and surface chemistry to target individual cells to electrochemical microelectrodes with attachment robust enough to withstand vigorous washing with test solutions.

EXPERIMENTAL SECTION

Solutions and cell preparation

The standard cell bath solution consisted of (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, and 11 glucose, pH 7.2. We used a "high-K^+" solution to depolarize cells and trigger exocytosis that consisted of (in mM): 55 NaCl, 100 KCl, 5 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose, titrated to pH 7.2 with KOH. We tested electrodes with a ferricyanide solution that consisted of 1 mM $K_3Fe(CN_6)$ in 0.1 M KCl, pH 3.0.

Chromaffin cells were isolated from bovine adrenal glands as described previously[32]. We kept cells in culture in a humidified incubator at 37 °C with 5% CO_2 for up to five days in poly-l-lysine coated T25 culture flasks in Dulbecco's Modified Eagles Medium supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin. In preparation for an experiment, cells were detached from the flask with a vigorous wash of culture media, then spun down at 100 g for 4 min. The supernatant was discarded and the cells were suspended and triturated in 5 ml standard bathing solution followed by a second pelleting. The supernatant was again discarded and the cells were re-suspended in 1 ml standard bathing solution resulting in a typical cell density of ~2×10^6 cells/mL. We loaded 50 μl of the cell solution on the microchip device and waited for 5 min to allow cell settling. We then vigorously washed the device with standard bathing solution twice to remove unattached cells. Exocytosis was triggered immediately following the wash by adding 100 μl of the "high-K^+" solution.

In later experiments we used an alternative culture method to make it easier to detach cells from the flask and to reduce cell clumping to make it easier to load individual cells on electrodes. Cells were cultured in Hibernate A media with calcium (BrainBits LLC, Springfield, IL, USA) in a refrigerator (4°C) and used 1~6 days after preparation as described in the previous paragraph.

Cell viability measurements were carried out as previously described[33]. Trypan blue solution (0.4%, Sigma, St. Louis, MO, USA) was mixed with regular chromaffin cell medium at a ratio of 2:3. Following cell loading and wash, 50 μl of this solution was added to the device. After allowing 5 min for staining, the solution was removed and replaced with 50 μl of fresh standard bath solution. Cells were then inspected under the microscope with dead cells exhibiting blue staining.

Device fabrication

Miroscope slides (25 × 75 × 1 mm, Fisherbrand, Fisher Scientific, Pittsburgh, PA, USA) were used as a transparent substrate whereas DLC:N was used as the electrode material.

Deposition and patterning of DLC:N/ITO films

DLC:N was deposited on top of transparent Indium-Tin-Oxide (ITO, 15–30 nm thick) because the resistivity of DLC:N is high (~1Ωcm)[9]. ITO-coated glass slides were purchased from Sigma-Aldrich (St. Louis, MO USA) and were cleaned with acetone, methanol and deionized water. The slide was blow dried with air and left on a hot plate for two minutes to remove the moisture. DLC:N was subsequently deposited on ITO surface using magnetron sputtering (ATC 2000-V, AJA international Inc., North Scituate, MA, USA) with 300 watts DC power, a pressure of 2 mTorr, Ar flow of 10 sccm, N_2 flow of 10 sccm and a temperature of 400°C. The deposition rate was ~2 nm/min and film thicknesses of ~40 nm were used for transparent electrodes with the microwell approach, whereas thicker (~120 nm) films were necessary for Teflon-insulated devices to prevent etch-through of the DLC:N film while patterning Teflon AF[29]. The sheet resistance of DLC:N on ITO was

measured using a four-point probe (S302, Lucas Labs, Gilroy, CA, USA) and was essentially identical to that of the underlying ITO film (70–100 Ω).

The DLC:N/ITO film was patterned using photolithography and wet etching into 40 conductive traces, 60 μm wide, with 3 × 3 mm connection pads arranged around the circumference of the slide. The process flow is depicted in Fig. 1. The DLC:N/ITO coated substrate was cleaned with 2-propanol, then sonicated for 10 min on each side in acetone (Branson Ultrasonic Corporation, model 1510, Danbury, CT, USA), then rinsed with deionized water and air-dried. Finally, the slide was cleaned with air plasma at the medium RF level for 1 min (plasma cleaner/sterilizer, PDC-32G, Harrick Scientific Corp., Pleasantville, NY, USA). Shipley S1813 positive photoresist (Rohm and Haas electronic materials, Philadelphia, PA, USA) was spin-coated (Single-wafer spin processor, Laurell Technologies Corp., North Wales, PA, USA) onto the DLC:N/ITO substrate at 2500 rpm for 1 min resulting in a thickness of ~2 μm. The coated DLC:N/ITO slide was pre-baked on a hot plate at 115 °C for 2 min. The photoresist-covered DLC:N/ITO slide was exposed with UV light (15 mW/cm^2) through a high-resolution (20,000 dpi) transparency mask (CAD/Art Services Inc., Bandon, OR, USA) and then developed in diluted M351 (M351: H_2O=1:4) (MicroChem Corp, Newton, MA, USA) for 1 min. Following development of the S1813 photoresist, the unprotected DLC:N film was etched with air plasma (PDC-32G, Harrick Scientific Corp., Pleasantville, NY, USA) at the High RF level for 15 min (40 nm films) or 18 min (120 nm films). Then the underlying ITO was wet etched using 0.2 M $FeCl_3$ in 6 M HCl for 30 min. Subsequently, the S1813 photoresist was removed using PRS-3000 stripper. Finally, the patterned device was rinsed with deionized water and dried using compressed air.

Patterning of microwells/working electrodes

We used the thick photoresist SU-8 2025 (MicroChem Corp, Newton, MA, USA) both to fabricate microwells and to insulate non-active areas of the conductive film. SU-8 was spin coated at 4000 rpm for 1 min to yield a film thickness of ~ 16 μm measured with a Tencor Alphastep 200 Profiler (San Jose, CA, USA). It was subsequently baked at 65 °C for 3 min and 95 °C for 5 min on hot plates. A second high-resolution (20,000 dpi) transparency mask was used to define a single 20 μm diameter microwell/electrode openings over each conductive trace as well as openings over each connection pad. Following mask alignment using a microscope, the photoresist was exposed with UV light (11.7 mW/cm^2). Then it was post-baked at 65 °C for 1 min and 95 °C for 5 min on hot plates. It was then developed with SU-8 Developer (MicroChem Corp, Newton, MA, USA) for 10 min with mild agitation. Finally, the device was baked on a hot plate at 150 °C for 30 min to seal small cracks on the surface of the SU-8 film.

Patterning of Teflon AF as an alternative insulating material

Teflon AF films were deposited and patterned as previously described[29]. An adhesion layer of FSM 600 (Cytonix, MD, USA), 5% by volume in ethanol, was spin-coated onto the device at 3500 rpm for 30 s and then baked on a hotplate for 10 min at 95°C. A 6% Teflon AF solution (Dupont, Wilmington, DE, USA) was diluted to 2% using FC-770 (3M, St. Paul, MN, USA). The 2% Teflon solution was spin coated on the device at 3000 rpm for 30 s followed by baking on a hot plate at 115°C for 15 min. Subsequently the temperature was increased to 225 °C for another 15 min and then to 300 °C for 1 hr. Following cooling, the Teflon-coated substrate was rinsed with acetone and heated to 95 °C for 2 min.

Two layers of S1813 photoresist were spin coated on the Teflon-coated device and baked as previously described. A second photomask determined the pattern of openings in the Teflon that serve as electrode/docking sites or connection pads. The photoresist was exposed

through the mask for 14 s and developed for 2 min with 1:4 diluted M351. Openings in Teflon were made using air plasma (PDC-32G, Harrick Scientific Corp., Pleasantville, NY, USA) at the High RF level for 5 min. Subsequently the S1813 photoresist was removed using PRS-3000 stripper, then washed with deionized water and dried with compressed air.

PDMS gasket

A gasket was fabricated to confine the drop of solution containing cells to the middle of the device where the 40 working electrodes are located. A 10:1 mixture of polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, Midland, MI, USA) monomer and cross-linking agent was poured into a 80 mm Petri dish, and degassed in a vacuum for 30 min to remove bubbles. Any remaining bubbles were removed with a needle, and then the mixture was cured at 80 °C in a conventional oven for 1 hr. The PDMS slab was cut into rings with an inside radius of 4 mm and an outside radius of 9 mm. The gasket was then placed at the appropriate location at the middle of the chip and then the assembly was treated with air plasma for 1 min at the medium power setting to enhance the seal of the gasket to the chip. This step also cleaned the electrode surface.

Surface modifications to enhance cell targeting to microwells/electrodes

We modified a recently described technique to graft cytophobic poly(ethylene glycol) on the surface of the SU-8 film with the aid of a poly(dopamine) adhesion layer[34]. Dopamine was adsorbed onto the surface of the SU-8 without entering the microwells using a stamping approach. Filter paper (Quantitative Q8, Fisherbrand Filter Paper, Fisher Scientific, Pittsburgh, PA, USA) was cut into ~ 7 mm diameter pieces and soaked in a solution containing 2 mg/ml dopamine hydrochloride in 10 mM Tris-HCl, pH 8.5. Two layers of the wet filter paper were then placed on the middle of the chip where the working electrodes were located. The assembly was then sealed in an 80 mm Petri dish wrapped in Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL, USA) and left at room temperature for at least 4 hr or overnight. Following dopamine application, the filter paper was removed and discarded and the device was rinsed with deionized water and dried with compressed air. An adlayer of poly (ethylene glycol) was covalently grafted onto the poly (dopamine) film using a Schiff base reaction with amine-terminated methoxy-poly(ethylene glycol)[34] (mPEG-NH_2, Nanocs Inc, New York, NY, USA). A solution containing 5 mg/ml mPEG-NH_2 in 10 mM Tris-HCl, pH 8.0 was stamped onto the chip device for 30 min using filter paper following the same procedure described above. Finally, a drop of solution containing 0.0025% poly (L-lysine) was placed on the center of the device for 30 min at room temperature. The poly (L-lysine) adsorbs to areas not coated with poly (ethylene glycol), i.e., the bottom of the microwells, and thereby promotes adhesion of cells to the working electrodes[33]. Following the coating procedure the device was thoroughly rinsed in deionized water and dried.

Amperometric measurements and spike analysis

Amperometric measurements were performed using an EPC9 amplifier (HEKA, Lambrecht, Germany) as a two-electrode potentiostat held at a potential of 700 mV relative to an Ag/AgCl wire inserted in the solution that served as the counter/reference electrode. During recordings the microchip device was placed in a custom-built chamber that facilitated electrical connection from the head stage to the connection pads at the edge of the chip and allowed imaging of cells over the electrode using an inverted microscope. Amperometric signals were low-pass filtered with a cutoff frequency of 2.9 kHz and sampled at a rate of 10 ksamples/s. Amperometric spikes were analyzed using the software of Segura et al.[35] running on Igor Pro (Wavemetrics, Lake Oswego, OR, USA). Spikes were not included in the analysis if the peak current was less than 2 pA, had negative charges, multiple peaks or other irregular shapes, or 10~90% rise times of greater than 20 ms. Statistical comparison of

spike parameters between electrodes was carried out using ANOVA equality of several means test (http://home.ubalt.edu/ntsbarsh/Business-stat/otherapplets/severalmeans.htm).

RESULTS AND DISCUSSION

Overview of device design and fabrication

A summary of the fabrication process is depicted in Fig. 1. DLC:N was chosen as the electrode material because it is inexpensive, reasonably transparent, has excellent electrochemical properties[9] and promotes cell adhesion to the electrode surface[33]. DLC:N is deposited on top of the transparent conductor ITO in order to reduce the series resistance. The transparent DLC:N/ITO films are deposited on top of a glass slide substrate (Fig. 1a) in order to allow visualization of cells sitting on the electrodes using a conventional inverted microscope. The conductive films are patterned into 40 individual conductive traces using contact photolithography (Fig. 1b and Fib 2a,b). Then the conductive film is insulated with a thick (~16 μm) film of SU-8 photoresist. SU-8 is then patterned using a second photolithographic mask to make 20 μm -diameter openings that define the 40 working electrodes and also serve as microwell cell trapping sites (Fig. 1c).

Cells stick to an unmodified SU-8 surface upon random settling out of a cell suspension, and consequently movement across the surface into the electrode microwells is impeded. Carpeting the surface of the chip with a high density of cells to fill the electrode microwells is not desirable because each electrode may have more than one cell in the immediate vicinity and therefore record responses from multiple cells. Our goal is for only one cell to be near each electrode so that we can clearly resolve cell-to-cell variability in exocytosis. Therefore we graft a poly (ethylene glycol) film to the surface of the SU-8 to resist protein adsorption and cell adhesion.

It has recently been reported that oxidized dopamine forms a robust polymer film on virtually any surface, and poly (ethylene glycol) can be covalently grafted onto the poly(dopamine) film using a Schiff base reaction[34]. We modified this approach to stamp the surface of the SU-8 with dopamine (Fig. 1d) followed by mPEG-NH_2 (Fig. 1e) so the surface, but not the well-electrodes, are rendered cytophobic. We subsequently treated the devices with poly (L-lysine) to promote adhesion of cells to the electrode surface. Experiments with fluorescently tagged FITC-poly (L-lysine) demonstrate that the poly (L-lysine) adsorbs within the well-electrodes but not to the PEG-terminated SU-8 surface (data not shown). Thus the microwells not only promote gravity-assisted trapping of cells but also facilitate patterning cytophilic and cytophobic films in exact registry with the electrodes.

The well-electrodes are arranged in four sets of 10 electrodes near the middle of the glass slide (dashed oval of Fig. 2a expanded in Fig. 2b). The 40 conductive traces lead to connection pads arranged around the circumference of the slide (Fig. 2a). The slide was placed in a custom-designed holder, which facilitated connection of the potentiostat to each connection pad on the slide (Fig. 2c, upper arrow). Cells and solution were confined to the middle area of the chip using a PDMS gasket and the Ag/AgCl ground/reference electrode was placed in the drop of solution containing the cells (Fig. 2c, lower arrow). The apparatus was placed on the stage of an inverted microscope (IX-50, Olympus America Inc., Melville, NY, USA) to allow visualization of cells over the electrodes.

Electrochemical testing of working electrodes

We measured the specific capacitance of the pattered electrodes using Pulse software (HEKA, Lambrecht, Germany) by applying a sinusoidal voltage stimulus (frequency 1 kHz, peak amplitude 1 mV). The specific capacitance is ~ 15 $\mu F/cm^2$ for DLC:N electrodes at this frequency, which is comparable to values we measure with carbon fiber microelectrodes[36].

The standard deviation of the current during amperometric measurements was typically 1.6 pA for a bandwidth of 3 kHz, which is only ~ 30% larger than that reported for a much smaller (5 μm radius) carbon-fiber microelectrode[36].

We next characterized the electrochemical performance of electrodes using cyclic voltammetry with the test analyte potassium ferricyanide. Fig. 3 presents a sample cyclic voltammogram obtained from a 20 μm diameter DLC:N electrode at a scan rate of 10 mV/s. The voltammogram exhibits a sharp reduction transition, indicating an appropriately low series resistance.

The voltammogram exhibits a diffusion-limited current (i_{lim}) at cathodic potentials. The theoretical value for i_{lim} for a disk electrode on an infinite, insulating plane is given by (equation 5.3.11 of [37]):

$$i_{lim} = 4nFDCr$$

where n is the valence of the reaction (one in this case), F is Faraday's constant, D is the diffusion coefficient (~7.2×10^{-6} cm^2 s^{-1} for $Fe(CN)_6^{3-}$), C is the analyte concentration (1 mM), and r is the radius of the electrode (~10 μm). Under our conditions, the theoretical value of i_{lim} is 2.8 nA, whereas the measured value is ~2.3 nA. The agreement is within 20%, suggesting that perhaps the working radius of the electrode is slightly smaller than expected, or the approximation of our well-electrode device by a disk electrode on an infinite plane is inexact[38].

Capturing of single cells over electrodes

Our goal is for individual chromaffin cells to be targeted to well-electrodes whereas the area between electrodes has few cells so that background amperometric currents from catecholamine release from other cells is minimized. We loaded 50 μL of a cell suspension containing bovine adrenal chromaffin cells onto microchip devices and waited for 1 hr to allow cell settling. We then washed the device with standard bathing solution three times to remove unattached cells. Fig. 4a presents a sample image depicting 8 out of 10 DLC:N electrodes in an array occupied by single cells whereas the inactive areas of the chip insulated with SU-8 are virtually cell-free. The polydopamine/PEG coating was essential to allow washing away of cells from the SU-8 surface (data not shown). We conducted 28 similar cell-loading experiments and found the capture efficiency of single cells over each DLC:N electrode to be 75 ± 8% (mean + SD). Figure 4b presents the cell density on microwell electrodes versus the PEGylated SU-8 surface and clearly demonstrates specific cell targeting.

We have previously reported selective targeting of cells to DLC:N electrodes using Teflon AF as the cytophobic, insulating material[29]. Whereas we were able to achieve 66% targeting efficiency with this DLC/Teflon approach, we found that the cells are easily washed away from the electrodes with solution exchange. When we carried out the same solution exchange procedure used above, only 16 ± 1.1% of electrodes had an adherent cell (10 experiments). Thus the microwell and surface-patterning approach presented here is clearly superior to the DLC/Teflon approach by both enabling more efficient targeting of cells to electrodes, more complete wash away of cells between electrodes, and much more robust attachment of cells to electrodes which is essential for experiments that require solution exchange. It also is not dependent on the use of a particular electrode material since excellent results (64 ± 14% targeting efficiency, 17 experiments) were also obtained for Au electrodes.

Amperometric measurement of quantal catecholamine release from single trapped chromaffin cells

In order to assay cell viability on the electrodes we used trypan blue to stain dead cells. In three separate experiments we detected no stained cells on the electrode docking sites, although an occasional dead cell could be found elsewhere on the chip surface.

Chromaffin cells were triggered to release catecholamines by adding a high-K^+ solution which leads to cell depolarization and influx of Ca^{2+} through voltage-gated ion channels. Fig. 5a depicts a sample amperometric recording on a DLC:N electrode from a single bovine adrenal chromaffin cell (inset Fig. 5b) with spikes that are consistent with previous reports of quantal exocytosis. The time interval enclosed by the dashed rectangle in Fig. 5a is expanded in Fig. 5b. Most cells exhibited depolarization-evoked exocytosis. In a typical set of experiments we observed high-K^+-induced amperometric spikes from 19 out of 22 cells. This indicates that the cells in the well-electrodes are viable.

Electrode stability and re-use

It is desirable for the electrode arrays to be re-usable for high-throughput studies of exocytosis. We therefore carried out a series of experiments where we recorded amperometric spikes from the same set of four electrodes following four rounds of cell exchange. In between loading cells the device was cleaned with a solution containing 25% H_2O_2 and 50 mM KOH[10] followed by a rinse in deionized water. We analyzed 303 spikes from 16 individual cells using the software of Segura et al.[35]. Fig. 6 presents mean ± SEM of peak spike current (I_{max}), spike charge (Q) and spike duration (interval where the amperometric current exceeds 50% of the peak value, $t_{1/2}$) for each cell recording. Clearly there is no apparent loss of sensitivity of each of the four electrodes upon re-use and the difference between mean values of the spike parameters among the four electrodes is small and did not reach statistical significance (p values from ANOVA means equality test > 0.5). Pooling of all the recorded spikes from this set of experiments results in a mean Imax value of 18.1 pA, a mean Q value of 1.14 pC (or 5.86 amol/vesicle) and a mean $t_{1/2}$ value of 39.1 ms. The values of Q and $t_{1/2}$ are within the range typically reported for bovine adrenal chromaffin cells using carbon-fiber electrodes[1, 2, 32, 39]. Cell-to-cell variability in spike parameters is comparable to that found with carbon-fiber electrodes where the standard deviation of Q is comparable to the mean value (coefficient of variation ~1) [40]. This variability means that many experiments need to be carried out to detect small changes in spike parameters resulting from an experimental manipulation and highlights the value of high-throughput approaches to measure quantal exocytosis.

CONCLUSION

We have developed an effective cell-targeting device suitable for high-throughput amperometric measurement of quantal exocytosis from individual cells without the need for microfluidic forces to be applied to the cell. Since single cells are targeted to electrodes without nearby extraneous cells, individual cell responses can be unambiguously recorded. Vigorous solution exchange can be carried out without displacing cells from the electrodes. The device can be cleaned and re-used multiple times without significant degradation of performance.

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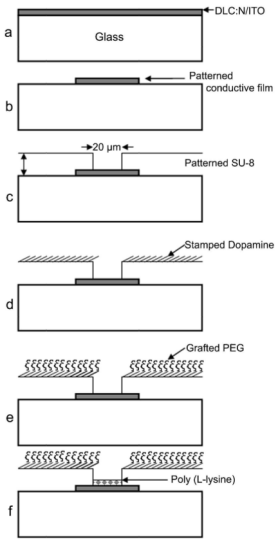

Fig. 1.

Process flow for microfabrication of electrochemical microelectrode array. (a) A thin semi-transparent DLC:N/ITO film is deposited on top of a glass slide substrate. (b) The film is patterned into 40 individual conductive traces using contact photolithography and etching processes. (c) The conductive film is insulated with a thick (~16 μm) film of SU-8 2025 photoresist. The SU-8 is then patterned using a second photolithographic mask to make 20 μm -diameter openings that define the 40 working electrodes and also serve as microwell cell trapping sites. (d) The surface of the SU-8 is stamped with oxidized dopamine to form an adhesive film. (e) mPEG-NH_2 is stamped on the surface and reacts with the polydopamine to form a cytophobic PEG surface. (f) A solution containing poly (L-lysine) is applied to form a cytophilic coating on the electrode surface.

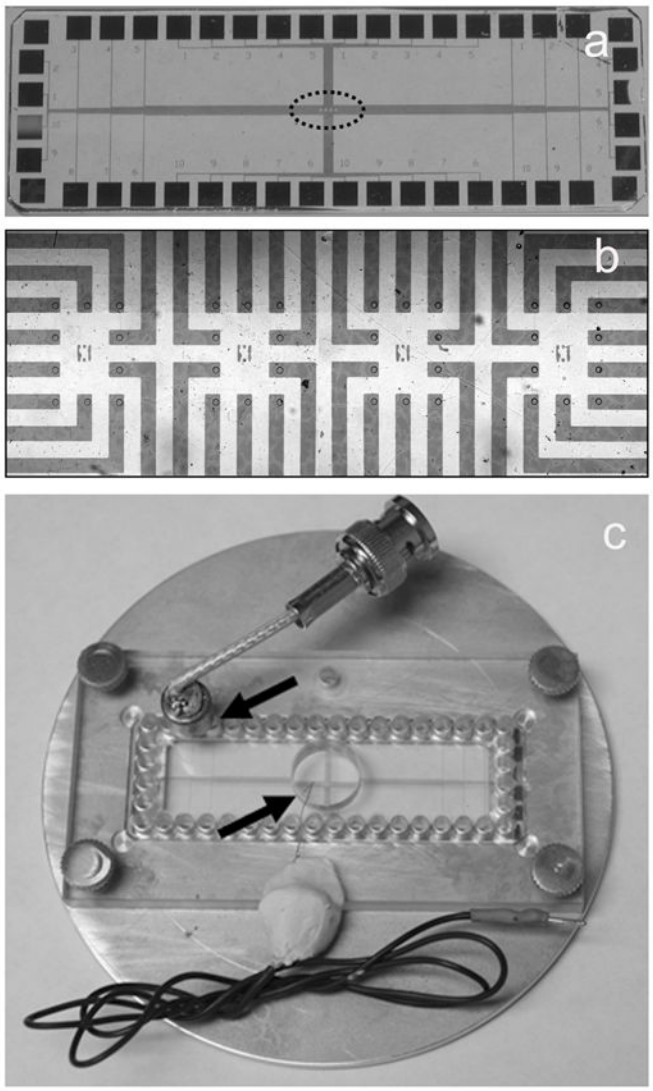

Fig. 2.

Photos of device and chip holder. (a) Photo of device on glass slide substrate. The 40 squares around the perimeter are connection pads whereas the working electrodes are in the center (dashed oval). (b) Expanded view of the 40 working electrodes, which are arranged in 4 sets of 10. Each conductive trace is 60 μm wide. (c) Photo of chip holder. The removable plastic cover has 40 holes to facilitate connection of amplifier to the connection pads (upper arrow). Cells and solutions are placed in the center of the device as is a Ag/AgCl reference electrode (lower arrow). The round aluminum bottom inserts into the microscope stage to allow viewing of cells.

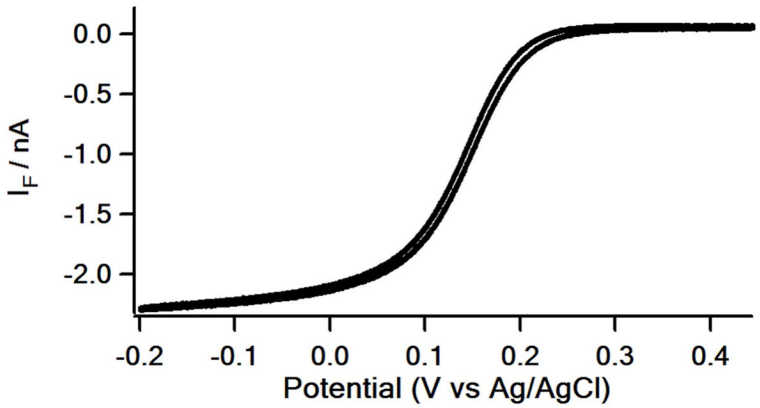

Fig. 3.

Cyclic voltammogram with test analyte (1 mM $K_3Fe(CN)_6$ in 0.1 M KCl, pH=3, scan rate 10 mV/s). The DLC:N/ITO working microelectrode had a radius of ~10 μm.

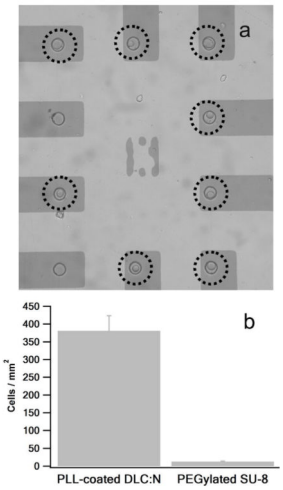

Fig. 4.

Individual chromaffin cells are targeted to electrochemical microelectrodes in an efficient manner using the microwell/surface patterning approach (a) Typical photomicrograph illustrating that 8 of 10 DLC:N electrodes (dashed circles) are occupied by single cells following cell loading and washing. (b) Bar graph quantifying the density of cells on poly (L-lysine) coated DLC:N electrodes versus the density of cells on the PEGylated SU-8 surface (mean ± SD).

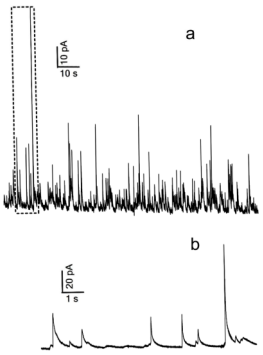

Fig. 5.

Typical recording of amperometric spikes reflecting quantal exocytosis from an individual cell. Exocytosis was stimulated with a high-K^+ solution. (a) A sample trace of quantal catecholamine release from single bovine adrenal chromaffin cell on a DLC:N electrode (b) Expanded view of the amperometric spikes within the dashed box in part (a).

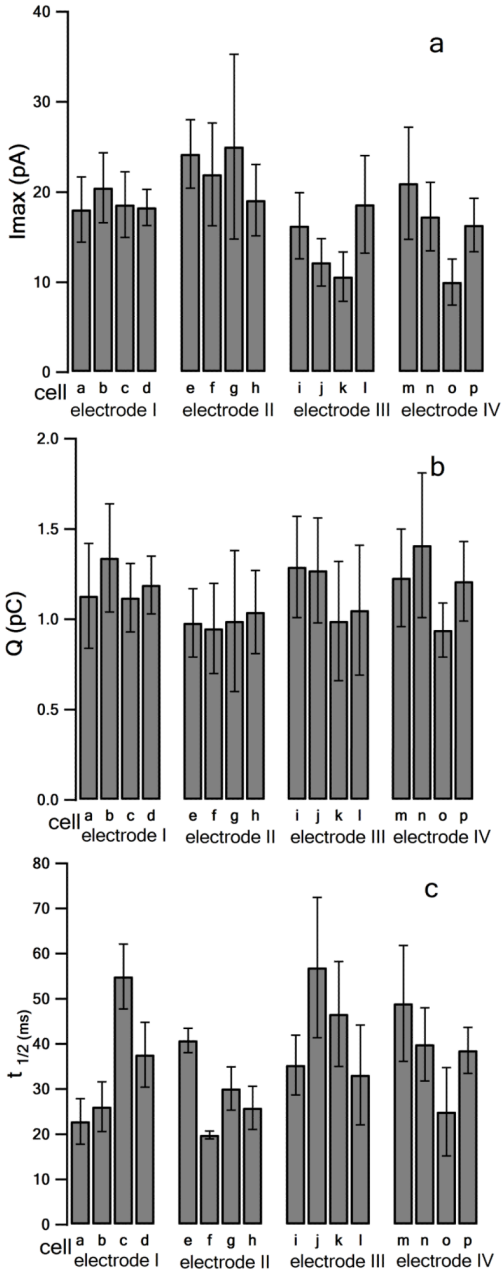

Fig. 6.

Electrodes can be cleaned and used again without changing spike parameters. In addition, the sensitivity of different electrodes to measure spike characteristics is similar. Amperometric spikes were recorded from the same four electrodes during four sequential rounds of cell experiments. The device was cleaned between loading each batch of cells.