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INVITED REVIEW

Surface-modified CMOS IC electrochemical sensor array targeting single chromaffin cells for highly parallel amperometry measurements

Meng Huang[1] · **Joannalyn B. Delacruz**[1] · **John C. Ruelas**[2] · **Shailendra S. Rathore**[1] · **Manfred Lindau**[1]

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Abstract Amperometry is a powerful method to record quantal release events from chromaffin cells and is widely used to assess how specific drugs modify quantal size, kinetics of release, and early fusion pore properties. Surface-modified CMOS-based electrochemical sensor arrays allow simultaneous recordings from multiple cells. A reliable, low-cost technique is presented here for efficient targeting of single cells specifically to the electrode sites. An SU-8 microwell structure is patterned on the chip surface to provide insulation for the circuitry as well as cell trapping at the electrode sites. A shifted electrode design is also incorporated to increase the flexibility of the dimension and shape of the microwells. The sensitivity of the electrodes is validated by a dopamine injection experiment. Microwells with dimensions slightly larger than the cells to be trapped ensure excellent single-cell targeting efficiency, increasing the reliability and efficiency for on-chip single-cell amperometry measurements. The surface-modified device was validated with parallel recordings of live chromaffin cells trapped in the microwells. Rapid amperometric spikes with no diffusional broadening were observed, indicating that the trapped and recorded cells were in very close contact with the electrodes. The live cell recording confirms in a single experiment that spike parameters vary significantly from cell to cell but the large number of cells recorded simultaneously provides the statistical significance.

Keywords Amperometry · Biosensor · Shift electrode · Cell trapping · Post-fabrication · High throughput · On-chip recording

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✉ Manfred Lindau
ml95@cornell.edu

[1] School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853, USA

[2] ExoCytronics LLC, 1601 S Providence Rd, Columbia, MO 65211, USA

Introduction

Neurotransmitters are released into the extracellular space in packages by the fusion of secretory vesicles with the plasma membrane, a process known as exocytosis [24]. Modulation of this process is an important drug target and crucial for molecular manipulation [44]. A narrow nanometric fusion pore forms between the secretory vesicle and its docking site at the cell membrane in the initial stage of exocytosis with the help of soluble N-ethylmaleimide sensitive fusion protein attachment receptor (SNARE) complexes, followed by the potential expansion of the pore and accelerated release of its content to the extracellular environment [20, 29, 50]. Amperometry is often used to measure catecholamine, a group of neurotransmitters including epinephrine, norepinephrine, and dopamine (DA). Upon reaching a polarizable electrode held at 700 mV against an Ag|AgCl reference electrode, catecholamine molecules are oxidized, transferring two electrons per molecule to the electrode. The resulting Faradaic currents can be detected and show characteristic amperometric spikes for each exocytosis event. The amperometry measurement provides precise details about the released neurotransmitters in a single quantal event. In chromaffin cells, a "foot" signal preceding the onset of an amperometric spike indicates the slow leakage of catecholamine out of the early fusion pore [10, 20]. The subsequent amperometric spikes correspond to the expansion of the fusion pore and rapid flux of molecules

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into the extracellular media [2, 29]. Amperometry reveals the release kinetics on the level of a single exocytotic event. It has been discovered by amperometry that the antihypertensive agent hydralazine does not reduce the event frequency but slows the rate of catecholamine release with reduced quantal size [31]. On the other hand, the Parkinson's disease drug L-DOPA causes increase in quantal size and half width while they are reduced by reserpine in both PC-12 and MN9D cells [14, 40].

The properties of amperometric spikes vary from cell to cell, even from the same cell under the same condition [11]. Therefore, a large number of release events from a large number of cells must be measured and analyzed to achieve the statistical significance in order to quantify the effect of drugs on exocytosis events or study the mechanism of the formation of the fusion pore. Although conventional carbon fiber electrode (CFE) amperometry offers precise and low-noise recordings of exocytosis events, it only records a single cell per time and the cost of CFE and time consuming recordings limit the efficiency of the experiments. The emerging CMOS technology and microfabrication techniques have significantly improved the electrochemical sensing systems for various biomedical and biophysical applications, and a variety of CMOS-based bio-sensing devices have been developed recently [5, 16, 23]. Our lab previously demonstrated a high-throughput CMOS sensor array platform with 100 electrodes specifically designed and optimized for amperometry measurement and validated it with parallel cell recordings [3, 4, 26]. While the CMOS-based biosensors significantly facilitate the electrochemical detection, targeting of the cells to the electrode sites for efficient measurement was still a challenge. Because the electrodes on a CMOS device only cover a fractional area on the entire device surface and cell density must be limited to avoid cell clumps, cells may not settle directly on an electrode and the released catecholamine may travel a long distance before detected. When cell density is too high, an electrode may record events from multiple cells if cells are clustered in its vicinity. Recently, SU-8 microwell structures have been used to trap single cells on passive electrochemical sensor devices [1, 30]. Here, we present the implementation of modified surface chemistry and developed a simple and time-saving cell trapping technique on active CMOS devices. Localization of chromaffin cells directly on or in close vicinity of the electrodes significantly reduces the diffusion distance and therefore time for catecholamine molecules to be oxidized. The single-cell trapping microwell also minimizes the interference from activities of other cells nearby. The modified surface structure also provides additional insulation for the CMOS circuit to avoid any leakage currents, decreasing the output noise level. The application of this device will dramatically facilitate the study of exocytosis events and drug modulation with reliable amperometry measurement from chromaffin cells, dopaminergic neurons, and other cell types.

Material and methods

Amperometry sensor array fabrication

The CMOS sensor array integrated circuit (IC) devices were fabricated at MOSIS through the ON Semiconductor C5F/N process as previously described [4, 26]. The exposed electrodes are originally covered by AlCu contacts because the fabrication process prohibits the use of polarizable electrode material such as Pt. However, AlCu contacts are not polarizable and induce low-resistance contact with the solution during measurement, leading to a high background current and fast consumption of the AlCu material. Therefore, Pt was deposited on the AlCu contacts as the active electrode material.

Post-fabrication to deposit Pt as a polarizable electrode material on the original Al contact was performed in house after receiving the chip using photolithography. To overcome the possible pattern distortion due to the edging effect during spin coating of photoresist on such a small device, a holder with the exact dimension to fit the CMOS die was fabricated by dry etching (Unaxis 770) on a silicon wafer, providing better handling and pattern transfer. The CMOS die was bonded inside the holder with epoxy (DOUBLE/BUBBLE Epoxy).

To avoid possible defects such as incomplete coverage of the AlCu contacts with Pt, a shifted electrode strategy was implemented to redefine the position and shape of the working electrodes, as shown in Fig. 1a. In addition, the insulation layer also provides a structure to trap cells on the chip, targeting one cell per electrode. The chip was spin coated with LOR 5A (Microchem) at 2000 rpm for 45 s followed by a soft bake at 180 °C for 5 min. It was then spin coated with Shipley S1813 at 4000 rpm for 30 s and soft baked at 90 °C for 1 min. A post exposure bake was performed at 115 °C for 1 min after exposure, followed by the development of the photoresist using MF321 for 1 min. The chip was descummed with YES Asher before metal deposition. A sputtering system (AJA International Inc.) was used to deposit a Ti adhesion layer (60 s, 10 nm) and Pt (500 s, 250 nm) with 400 W power on the AlCu contacts to achieve a uniform metal film as well as a good side wall coverage for electrical connection, which is crucial for the shifted electrode design. The lift-off process used Remover PG (Microchem) at 40 °C to dissolve LOR overnight. To fabricate the insulation layer and cell trapping microwells, SU-8 2025 (Microchem) was spin coated on the device at 4000 rpm for 1 min to achieve a thickness of 16 μm. The chip was soft baked at 65 °C for 3 min and then 95 °C for 5 min before exposure using a 350-nm filter. Subsequently, a post exposure bake at 65 °C for 1 min and then 95 °C for 5 min was performed before the device was developed in SU-8 developer (Microchem) for 6.5 min with mild agitation. The device was baked at 200 °C for 10 min to cure the cracking of the SU-8 layer during development. SU-8 as an insulation layer or mold for microfluidic devices is rarely patterned

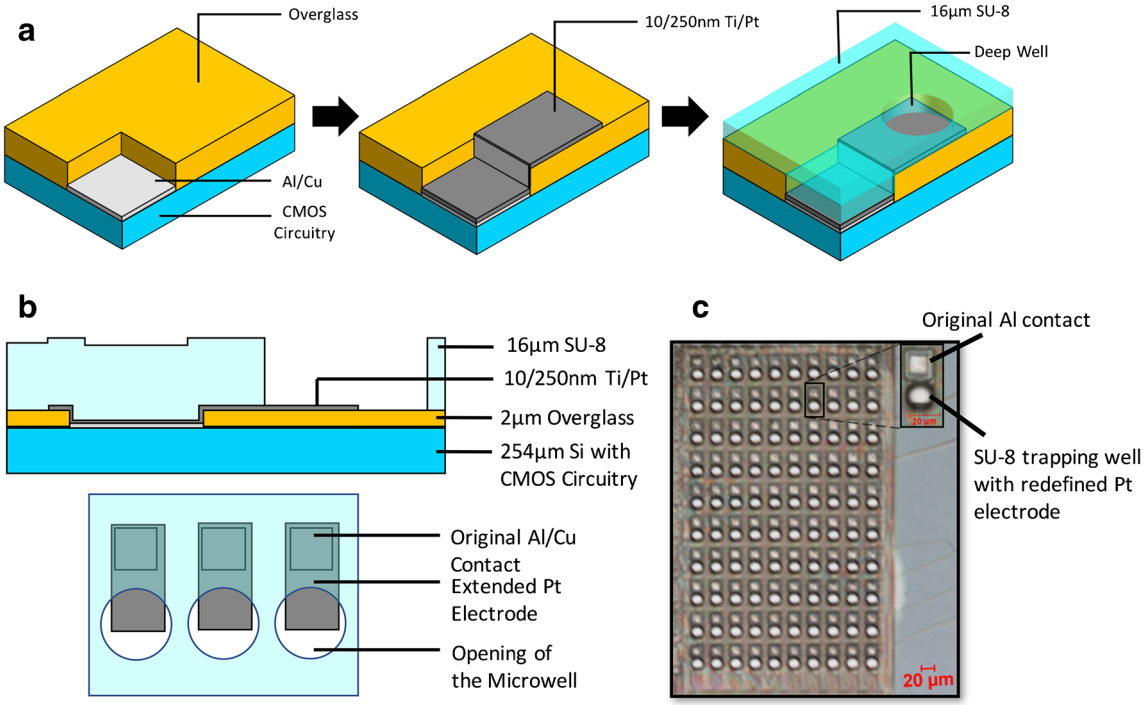

Fig. 1 **a** Post-fabrication flow of the shifted electrode design with SU-8 microwells. **b** Cross-sectional and top view of the schematic of the microwell structure. The dimensions of the microwell are designed to fit the size of a single bovine chromaffin cell. The dimensions for the cross-sectional image are not to scale. **c** Optical micrograph of the entire sensor array with the shifted electrode design and microwell structure. On the original AlCu contacts, Pt was deposited and further covered with SU-8 to prevent any risk of contacting with the solution

to features with high aspect ratio (defined as the depth divided by the width) because it is challenging to develop the photoresist homogeneously since the fresh developer may not enter the deep wells or channels efficiently due to its hydrophobic nature. In our case, the aspect ratio for the microwells is ~ 1.3, which is quite high considering the densely packed sensor array. To solve the issue, the chip was treated with a strong stream of oxygen plasma using Gasonics Aura 1000 resist stripper for 30 s after SU-8 development. The oxygen plasma helps removing any undeveloped SU-8 residual on the electrode to solve the development issue, as well as making the surface more hydrophilic [32]. About 1 µm of the SU-8 layer was etched during the final descum process. Figure 1b shows the cross-sectional and top view pictures for the 20 µm in diameter and 15-µm-thick cell trapping microwells. In the present design, the working electrodes in Fig. 1b cover only a portion of the bottom of the microwells, which was chosen to avoid the risk of conducting connections between two adjacent electrodes that could be present if the Pt lift-off is imperfect. If the Pt electrode would cover the entire well the adjacent Pt area would be very close, introducing be a risk that some adjacent electrodes may be shorted together after the lift-off process. The final structure of the entire sensor array is shown in Fig. 1c.

The die was attached and wire bonded on a side-braze dual in-line package (Addison Engineering, San Jose, CA, USA). Silicone (RTV 615, GE) was applied to insulate the device except for the center sensor array area to prevent the wires and pads contacting with the solutions.

Buffer and DA solutions

Standard extracellular solution contained (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, and 10 HEPES/NaOH with pH adjusted to 7.3 and the osmolality adjusted to 315 mmol/kg with glucose. The high K^+ stimulation buffer contained 105 KCl, 50 $CaCl_2$, 0.7 $MgCl_2$, and 10 HEPES/NaOH with pH adjusted to 7.3 and the osmolality adjusted to 315 mmol/kg with glucose. DA testing solution contained 420 µM DA in standard extracellular solution. The device was first covered with 100 µL standard extracellular solution, and 20 µL DA testing solution was injected from a pipette resulting in a final DA concentration of 70 µM.

Cell preparation and culture on the device

Bovine adrenal glands were collected from a local slaughter house and bovine adrenal chromaffin cells were prepared as previously described [35]. For same day measurements, a cell suspension was plated directly on chip. For longer culturing time, cells were cultured in flasks in the incubator at 37 °C and 8% CO_2 for up to 4 days and re-suspended for application to the chip. Twenty microliters of cell suspension in media with 10,000 cells was added on the device and cultured in the

incubator at 37 °C and 8% CO_2 for 2 h. Before the recording, the culture media was exchanged with 40 μL of standard extracellular solution. Stimulation of the cells was performed by injecting 80 μL of the high K^+ stimulation buffer, resulting in a final K^+_m concentration of 70 mM.

Sensor array preparation for cell trapping

The method described in [30] was modified to a simplified and time-saving protocol for cell trapping. Isopropanol and milli-Q water were used to rinse and clean the CMOS sensor. The sensor array was covered with 100 μL of 0.0025% Poly-L-Lycine (PLL) for 1 h and then placed in the incubator at 37 °C for 2 h. It was then treated with CO_2 gas flow and covered by 20-μL media for 4 h to ensure complete wetting of the microwells with the solution.

Biosensor recording and data acquisition and analysis

A grounded Ag|AgCl reference electrode was inserted into the buffer solution before the recording. On-chip electrodes were set at 700 mV against the reference electrode. Clock signals and power supplies were applied to operate the chip. A sampling rate of 2000 S/s was used for each electrode for both the DA and cell recordings. The analog outputs were converted to digital signals through NIDAQ PXIe-6368 (National Instruments, Austin, TX) and collected by the computer using Igor Pro 6 (Wavemetrics). Raw data was then demultiplexed based on the sampling rate and filtered by 5 pass binomial smoothing in Igor Pro 6. Amperometry data analysis for live cell recordings was performed using IGOR-based Quantal Analysis software [33].

Random walk simulation of DA diffusion

Random walk simulations for DA diffusion on the electrode array were performed based on a user-written procedure in IGOR Pro 6 described previously [21, 50]. The diffusion coefficient of DA molecules was taken to be 6×10^{-6} cm^2/s, the typical diffusion coefficient of DA in free solution [19]. The total simulated volume was formed by the 450 μm × 270 μm surface of the chip with the electrode geometries from the chip as shown in Fig. 1 and a 50-μm-high solution covering the chip surface. Since the depth of the microwells was small compared to the lateral dimensions, the microwell structure was not included in the simulation set-up. During the simulation, a total of 1 billion molecules was injected at the position where in the real measurement, the first current response occurred and this time point was defined as $t = 0$. When a molecule made contact with an electrode, it was counted and removed from the simulation, corresponding to the oxidation and consumption of the molecules in the experimental measurement. The number of detected molecules in 10-ms time intervals was taken as a measure of the amperometric current for a given electrode.

Results

DA diffusion on the chip surface

For a basic response test of the chip, DA solution in standard extracellular buffer was applied from a pipette onto the chip for 70 μM final concentration and the currents reported by the sensor array are shown in Fig. 2a. The colors indicate for each pixel the measured current amplitude as shown on the side legend. Labels above each image indicate the time after DA injection. The DA molecules first appeared on the bottom right region of the sensor array and then diffused to other parts of the sensing area. The chip clearly displayed the diffusion path of DA and revealed the relative DA concentrations through current levels at each electrode. Figure 2b shows random walk simulation results for the DA molecules. The start point of the simulation was chosen to be located at the electrode position at which the first DA signal appeared in the experiment. Although the concentration profile of DA on the chip is complicated, the measured current represents the local concentration at the surface of each electrode. The concentration profiles obtained in the simulation closely matched the experimentally measured profiles and the diffusion time course. Using the midpoint (half maximal) current levels on the surface plane along the vertical direction as an average diffusion distance estimate, the simulation results showed ~ 25% smaller average diffusion distances of the DA molecules in the simulation compared to the measured data. The difference may be due to the shallow height of the solution layer (50 μm) in diffusion model used for the simulation and also possible directed flow induced by the injection. In the simulation, the confinement in the shallow volume leads to a higher probability of a molecule to hit an electrode and be oxidized, leading to a reduced apparent diffusion rate. After 2 s, about 65% of the simulated molecules had been oxidized in the simulation. Nevertheless, the close similarity of the measured and simulated diffusion patterns validates the function of the device incorporating the SU-8 microwell structure for measuring oxidizable molecules.

Cell trapping in SU-8 microwells

To examine the cell trapping efficiency of the device, the device was first treated with CO_2 gas flow and covered by 20-μL media for 4 h. CO_2 treatment was performed to replace the air inside the microwells with CO_2 gas, which dissolves better in aqueous solutions, reducing the appearance of air/gas bubbles. Further wetting with a small amount of media or buffer solution also improved the wettability of the

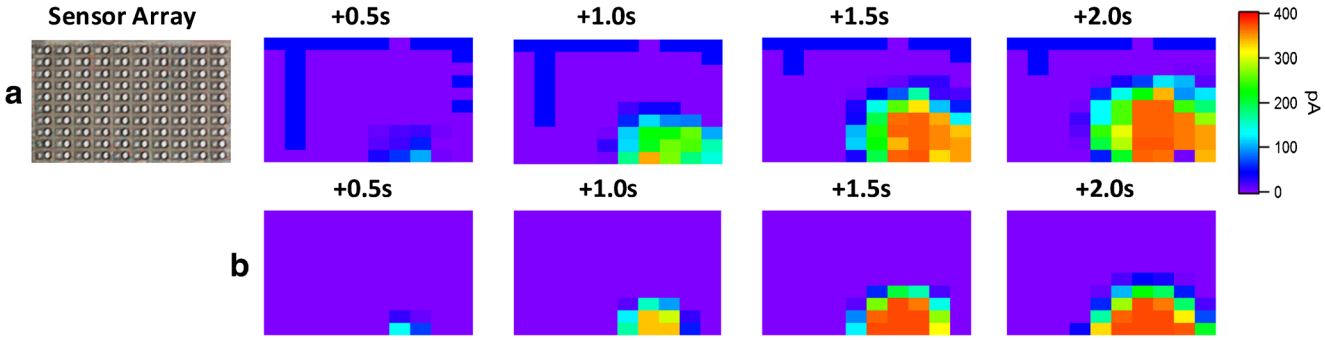

Fig. 2 **a** Visualization of DA diffusion on the surface of the chip. The colors for the pixels are based on the current levels measured at each electrode, as depicted on the legend. **b** Simulated results for DA diffusion on the chip surface. The colors for the pixels are normalized based on the number of DA molecules reaching the electrodes

microwells. Bovine chromaffin cells were cultured in media on chip for 2 h before washed with standard extracellular buffer. Only cells firmly attached to the chip surface remained after the wash. Totally, 37 out of 100 microwells on the device in Fig. 3 trapped cells inside, as marked with blue circles. Among the microwells with cells trapped, 32 microwells had a single cell inside while 3 microwells had two cells trapped, and 2 microwells had three cells trapped, resulting in a single-cell trapping efficiency of 32%. No cells were attached on the SU-8 layer in the sensing area after the wash. The average targeting efficiency from 14 independent experiments was 22 ± 7% (mean ± SD).

PLL promotes the adhesion of cells on the coated surface. It is interesting to note that few cells were seen being attached to the SU-8 covered areas on the chip surface. One possible reason could be that the adsorption of PLL on the overglass and the Pt electrodes of the chip is very high, but much less efficient on SU-8 due to its hydrophobic nature. The additional heating at 37 °C in the incubator further weakens the bonding between PLL and SU-8. Thus, cells may not attach easily on the SU-8 layer due to its hydrophobicity and the described treatment, leading to selective cell adhesion at the electrode sites of the surface-treated sensor array. Another possible explanation is that the microwells trapping cells cover a large area of the chip surface and therefore, there is less chance that the cell will be settled on the SU-8 layer. It is noteworthy that the depth of the microwells and the aspect ratio play a significant role for cell trapping efficiency. For bovine chromaffin cells, devices with microwell depth below 12 μm or an aspect ratio below 0.6 did not show significant cell trapping in the microwells. Once a well is occupied by a cell, there is a possibility that another cell will settle down on top of it, which is not ideal for long culture times. To remove overlapping cells, the chip was washed with fresh media a few times during the culture. The efficient targeting provides great potential to facilitate the parallel recording of single-cell amperometry measurement with precise and reliable results.

Live chromaffin cell recordings

To demonstrate the viability of live cell measurements, a bovine chromaffin cell suspension was plated on the device and allowed cells to settle for 2 h in the incubator. After washing with the standard extracellular buffer to remove unattached cells, the remaining cells were stimulated with high K^+ stimulation buffer to trigger exocytosis events, which were recorded by the data acquisition system at 2 kS/s. Figure 4 shows the amperometry recordings for four selected cells. Individual amperometric spikes are shown on expanded scale in the framed boxes. Microscope images of the individual trapped cells generating the corresponding responses are also shown. Ten seconds after the beginning of the recording, the cells were triggered with the stimulation buffer for exocytosis events and each amperometric spike indicated a quantal

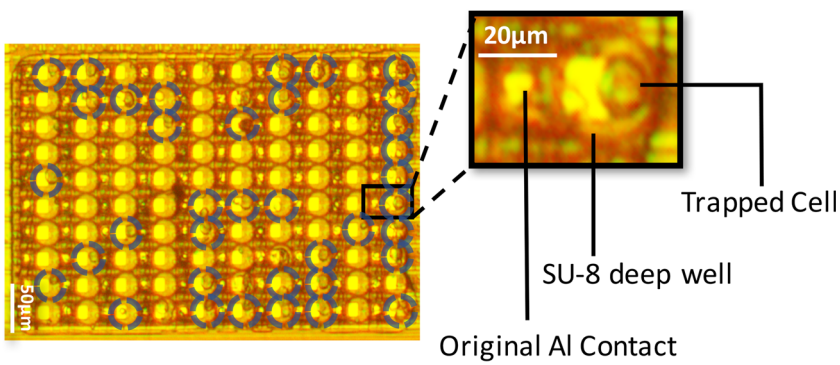

Fig. 3 Micrograph of the sensor array with loaded cells. Cells are trapped in 37 microwells with 32 of them occupied by a single cell. No cells are attached on the SU-8 layer within the sensing area

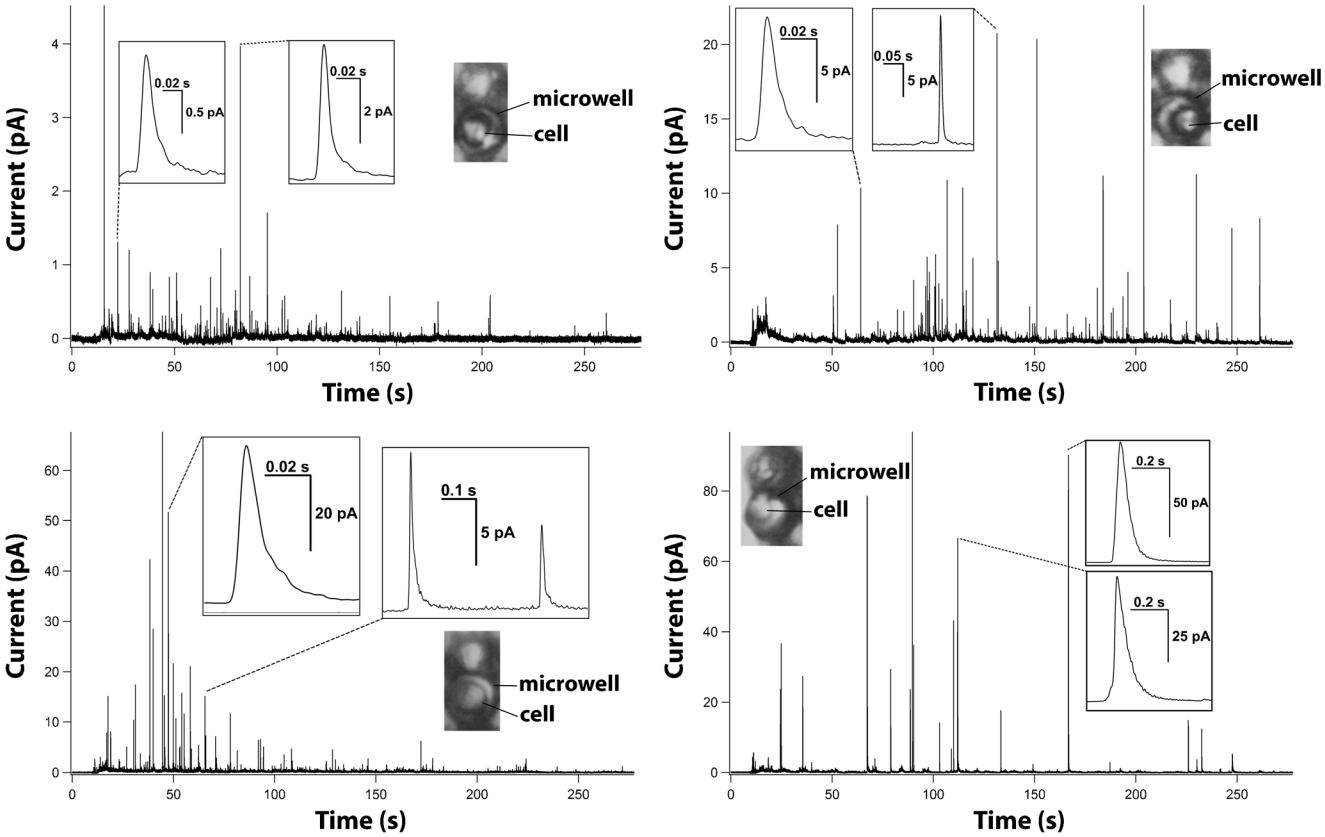

Fig. 4 Amperometry recordings for live chromaffin cells on chip. Four selected traces for four cells are demonstrated. Example amperometric spikes are zoomed in the boxes. The cells corresponding to the four traces are shown in the microscopic images. Cells were settled down individually in the wells and directly on the electrodes during the recording

release event. The baseline was stable with an average noise level of 150 fA at 2 kS/s. Based on our estimation, exocytosis events down to approximately 7000 molecules should be detectable. A very small amperometric spike is shown in Fig. 5, which has a quantal size of 3.334 fC or approximately 10,400 molecules, which is very close to the estimated limit and resembles the average quantal size measured in dopaminergic neurons using carbon fiber amperometry [43]. The analysis of the top right trace in Fig. 4 reveals 66 clear events with 31 of them having a foot signal. Events with a maximum current below 500 fA were not included in the analysis. The mean half width of all events was 9.76 ± 0.63 ms (mean ± SEM), which is typical for an exocytosis event close to the electrode [41]. Mean quantal size, mean maximum current, mean foot duration, and mean foot amplitude for this cell were 0.114 ± 0.02 pC, 4.35 ± 0.63 pA, 14.69 ± 4.54 ms, and 0.34 ± 0.05 pA (mean ± SEM), respectively.

Events from 28 cells were analyzed with the same approach. The results from 5 individual cells and the mean values from all 28 cells are shown in Fig. 6. Mean spike parameters for each individual cell/electrode are shown as white bars. The error bar is in SEM. The numbers in parenthesis indicate the number of events analyzed. Six hundred forty-nine events from 28 cells were detected in total, and the quantal sizes ranged from 0.0033 pC (~ 10,400 molecules) to 9.48 pC (~ 29.6 million molecules) but based on the 500 f. amplitude threshold only

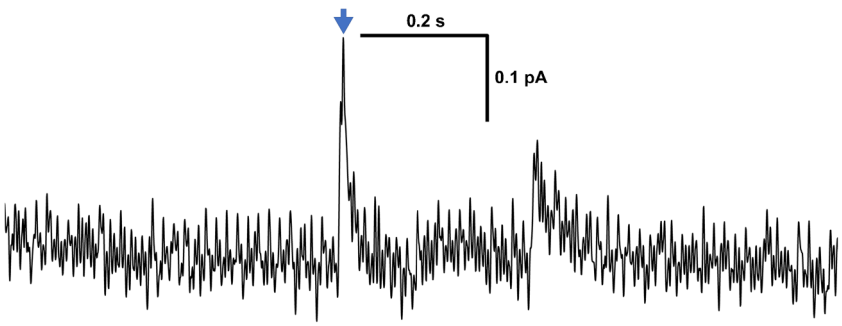

Fig. 5 A small amperometric spike (showing with a blue arrow) with a quantal size of 3.334 fC or approximately 10,400 molecules. The quantal size is close to the detection limit of the sensor array, which is approximately 7000 molecules

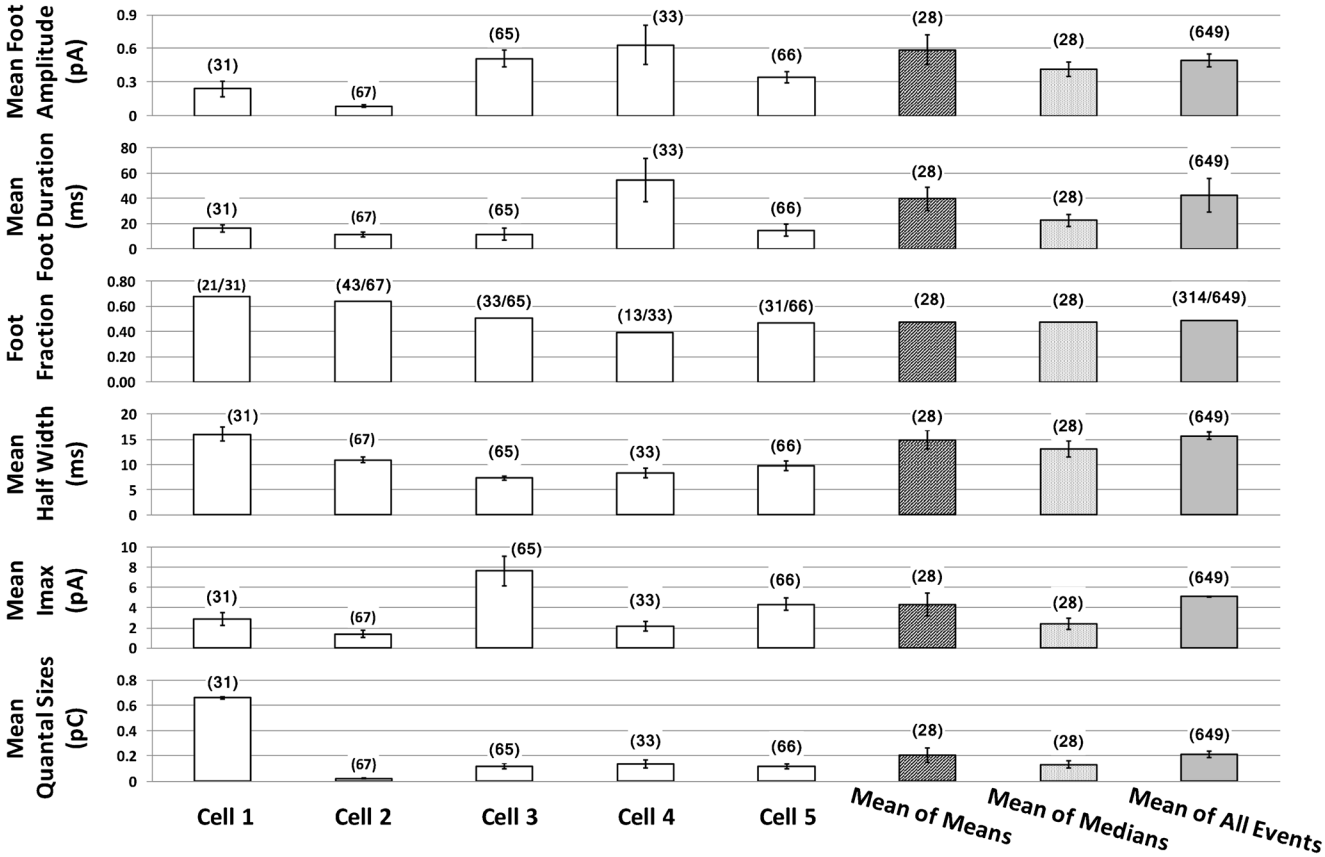

Fig. 6 Amperometric parameters for cells/electrodes with error bar in SEM. Each column indicates one single cell. The parameters for five selected cells are shown in the chart for comparison. The number in the parenthesis is the number of events analyzed. Data from 28 cells and 649 events are analyzed in total. Standard method is to calculate the mean of cell median values (patterned with diagonal lines). For comparison, mean of cell mean values (patterned with dots) and mean of all events (gray) are also shown. Note that for mean of means and mean of medians "n" is the number of cells

events with quantal size > 0.01 pC were included in the statistical analysis. The small mean half width shows that there was no significant diffusional broadening due to the long traveling distance of released molecules to the electrode [21, 41], and therefore, the cells were in close proximity to the electrodes ascribed to the cell trapping microwells. It is apparent that spike parameters vary significantly from cell to cell, and therefore, it is of great importance to achieve the statistical significance by collecting data from a large number of cells [11], which again emphasizes the potential of the surface-modified sensor array for amperometry recordings characterizing modulation of release by specific drugs and molecular manipulations.

Discussion

CMOS-based sensor arrays with on-chip amplifiers greatly facilitate the electrochemical measurement in multiple fields, especially for measuring activities from neurons, embryoid bodies, or tissues as [25, 32, 34] they are large and occupy multiple electrodes for recording. However, for single-cell measurements, the recordings are usually less successful because it is not trivial to target cells individually to the electrodes. Cells are usually plated on the device and targeted by chance [26]. It is possible to use a glass micropipette to precisely place a cell on the electrode [27], but it requires intensive labor and only one cell can be placed each time.

Microelectrode arrays have been fabricated in recent years to measure quantal release events [9, 13, 18, 36]. Some targeted at amperometry in combination with random walk simulation and total internal reflection fluorescence (TIRF) microscopy [21, 27, 50]. Although the devices enable amperometry and TIRF imaging simultaneously, these arrays rely on external amplifiers and are not suitable for large-scale parallel recordings of many cells. Two approaches were developed to multiplex the outputs and reduce external connections [49]. However, the approaches still require external amplifiers and due to the need of external connections, the size of the array is not scalable. The device we demonstrated in this work features on-chip amplifiers and multiplexing architecture with 100 electrodes with cell trapping wells and is suitable for the application to study the quantal release events from a large scale of samples. We are currently extending the device to 1000 electrodes, a number that cannot be achieved using external connections for each electrode.

Various materials have been tested as electrodes for amperometry, including diamond-like carbon [18, 27], indium tin oxide [27, 45], boron-doped diamond [8, 42], and graphitic electrodes embedded in a diamond substrate [8, 36–39]. Each of these materials has their unique property for measuring quantal release events. While most of these materials cannot be straightforwardly deposited on a CMOS device as the device is easy to be damaged under various conditions, some such as boron-doped diamond have been successfully integrated on the CMOS electrode arrays with a bonding interlayer [22]. Conducting polymers are also potential candidates for electrode materials. PEDOT:PSS microelectrode fabricated using the lithography techniques was successfully used for amperometry measurements with chromaffin cells [47]. This provides the potential of incorporating different kinds of materials on the CMOS chip as electrodes to characterize and take advantages of their properties. Other molecules, such as glucose and lactate, can also be detected by binding glucose oxidase and lactate oxidase on the Au electrodes [16]. It is possible to immobilize oxidases and enzymes on the electrode for our device to measure various kinds of cells and activities.

Cells that are trapped at the bottom of the microwells will not be easily washed away and therefore enable the recording one cell per electrode. However, the wash is performed manually and a harsh wash may still remove the cells from the wells if not controlled properly. A cell trapping microfluidic device was fabricated to automatically target individual cells to the electrodes [17]. A two-layer structure for microfluidic device was also developed on a silicon substrate [15], which makes it possible to fabricate microfluidic devices on CMOS chips. Such microfluidic device may enable standard fully automatic protocols for high throughput and high reproducibility.

In this work, we have presented a CMOS-based electrochemical sensor array for amperometry measurement with modified surface structure for cell trapping. We have shown that the surface SU-8 microwell structure can provide a better insulation for the circuitry as well as enable cell trapping inside the microwells. The DA diffusion experiment revealed that the hydrophobic nature of the SU-8 layer could be chemically and physically modified through oxygen plasma treatment and CO_2 gas flow for exchange of the air bubbles in the microwells for better wettability of solutions, and proved the sensitivity of the electrodes. For nine chips from the same post-fabrication batches without these treatments, none of the electrodes detected current signals while adding the dopamine solution, indicating that the oxygen plasma and CO_2 treatment were highly effective. The single-cell targeting efficiency for the cell trapping microwells was 22.2 ± 6.9% (mean ± SD), and no cells were observed on the SU-8 layer in the active sensing area. The trapped cells were in very close vicinity to the electrodes, resulting in rapid amperometric spikes, and no diffusion broadening due to the long travel distance of catecholamine was found. The ultralow noise level for the sensor array at 2 kS/s was ~ 200 fA, with an estimation of a quantal size resolution of ~ 7000 molecules. This resolution is sufficient to resolve quantal release events from dopaminergic neurons [43]. The live cell recording demonstrated that spike parameters vary significantly from cell to cell. Therefore, it is of great importance to collect a large number of data to achieve the statistical significance, and the presented CMOS-based sensor array can greatly improve the efficiency of amperometry measurement.

The SU-8 surface microwell structure and the cell trapping protocol we designed in this work, together with the CMOS biosensor, significantly simplify the single-cell amperometry measurement. Cell suspensions can be plated directly on the chip, and cells can be trapped automatically without any more manipulations. For each chip, it only takes about 10 min from taking the chip out of the incubator to finish the recording, and tens of cells, depending on the targeting efficiency, can be recorded in parallel.

In recent years, many dopaminergic drugs such as afobazole, citalopram, and bupropion have been studied in terms of secretion, the total amount of transmitter discharged after the stimulation [7, 12, 28, 46]. However, the effects of these drugs on the frequency, quantal size and kinetics of individual release events are unknown. Amperometry provides the technique of measuring neurotransmitter releasing kinetics for drug-treated cells. The mechanism of the antihypertensive agent hydralazine was first explained in terms of exocytosis kinetics by amperometry and was found to slow the rate of catecholamine release from the release events but not the frequency of the exocytosis events [31]. Similar experiments can be performed on other drugs, which affect catecholamine release to reveal the effect of the drugs on the single cell and single release event level. The CMOS sensor array we developed in this work can greatly improve the efficiency of these experiments.

As shown in Fig. 5, individual release events with a quantal size of ~ 10,000 molecules, which is typical for dopaminergic neurons [43] are clearly detectable, opening the possibility to apply the CMOS chip design presented here for measurements of quantal release from dopaminergic neurons. However, the average half width of amperometric spikes from rat dopaminergic neurons is ~ 100 μs [43]. To resolve the kinetics of such release events, a sampling rate of 20–40 kS/s and correspondingly shorter integration times will be necessary and the increased sampling rate will increase the noise level. At 2 kS/s, the RMS noise is around 150 fA and the estimated noise levels at 20 and 40 kS/s will be 474.34 and 670.82 fA, respectively, as the noise is approximately proportional to the square root of the bandwidth [48]. Nevertheless, typical amperometric events from dopaminergic neurons will be clearly resolved because the peak amperometric currents reported for rat dopaminergic neurons are around 10–20 pA [43] and a noise level

< 1 pA will provide an excellent signal to noise ratio. The noise effectively determines the detection limit of charge with shorter events having larger amplitude for the same charge. The event illustrated in Fig. 5 has a long duration with a half width of about 11 ms but a small amplitude of approximately 0.25 pA. For the same quantal size of 10,000 molecules, an event with 100-μs half width would produce a 100 times larger amplitude of 25 pA, consistent with the range of 10–20 pA peak amperometric currents reported for CFE measurements on dopaminergic neurons [43].

Culturing neurons on a CMOS-based chip for 3 to 4 weeks has been reported [34] and will likely be feasible. For such neuron cultures, deep microwells will, however, not be incorporated. The SU-8 insulation layer will instead be replaced by a glass (SiO_2) insulation layer with a thickness < 1 μm [6] to allow the neurons to attach to the surface and also to have their release sites in direct contact with the electrodes. Neurons extend dendrites and axons over large distances, which are expected to extend over multiple electrodes. The electrode array design described here is scalable and a 1024 electrode design is currently in progress. We expect that the CMOS chip design with its excellent noise properties will make it possible to record simultaneously from multiple release sites from one or multiple neurons. The CMOS chip described here therefore provides a much better temporal resolution and in addition spatial resolution compared with the conventional CFE method and also compared to other possible CMOS chip designs capable of amperometry measurements [23] because the compact amplifier circuit we are using enables a much smaller pitch and therefore particularly high density of electrodes on the array.

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Compliance with ethical standards

Conflict of interest M.L. is a partner and J.C.R. an employee of the company ExoCytronics, which will develop and commercialize the microchip array platform technology presented here. ExoCytronics has won an SBIR phase I award from the NIH, which is supporting the development of amperometry/FSCV microchip technology.

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