LARGE-SCALE SCREENING OF OLFACTORY SENSORY NEURONS WITH AN INTEGRATED MICROFLUIDIC PLATFORM

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ABSTRACT

This paper describes a new platform for the capture, perfusion, and imaging of a large number of dissociated olfactory sensory neurons (OSNs) simultaneously. The platform consists of a fluid selection device integrated with a multiwell plate and a removable-top cell perfusion chamber bonded to an array of addressable cell-trapping microwells. Integration of a 24-well plate with our PDMS device allows fluids to be stored on-chip and eliminates the need for external reservoirs and tubing. The removable-top perfusion chamber facilitates cell seeding and enables cell retrieval post-imaging. Finally, an addressable array of microwells allows specific cells of interest to be located following calcium imaging and immunostaining.

KEYWORDS: Large-Scale Screening, Olfaction, Microfluidic Integration

INTRODUCTION

In the mammalian olfactory system, over a thousand different odorant receptor types are used in different combinations to sense odorants, with one odorant receptor expressed per olfactory sensory neuron (OSN) [1]. Previously, we developed a microfluidic platform for high-throughput calcium imaging of dissociated OSNs to enable visualization of rare OSN responses amongst thousands of cells [2]. In that system, the fluid valves were manually actuated, resulting in poor control. Additionally, as the cell chamber was permanently closed and did not contain landmarks, it was not possible to track and/or retrieve individual cells after imaging.

THEORY

We have integrated a 24-well plate with a PDMS device so that the wells of the 24-well plate act as open-well inputs of the PDMS device (Figure 1). This tubeless mode of fluid introduction is much more user-friendly than the cumbersome tubing used in traditional microfluidic systems (Figure 1). The bottom PDMS half contains 8 different valved stimulant lines and a buffer line, with valves placed at the junction between the stimuli lines and the buffer line to minimize dead volume (Figure 2). These PDMS layers consist of 45 micron-thick fluid and control layers, with a 10 micron-thick PDMS membrane sandwiched in between. A bottomless 24-well plate is attached to the top surface of the PDMS by modifying the bottom surface of the microplate with 2% aminopropyltriethoxysilane (APTES), followed by bonding it to the top surface of the PDMS device using oxygen plasma. Eight of the wells interface with separate stimulant lines, while the other 8 are connected together to serve as a large common buffer reservoir. An airtight lid with a single pressure line feeding into it allows all of the fluid samples to be pressurized simultaneously. Alternatively, fluid may be driven through the device by pulling with vacuum from the outlet. The pneumatic lines that actuate the valves are controlled by LabView software.

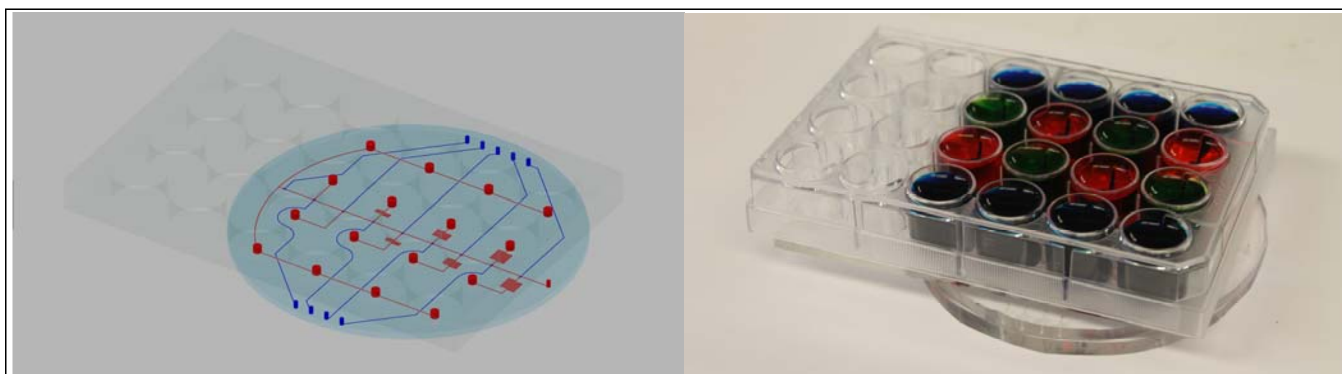

Figure 1: Schematic (a) and photograph (b) of the multiwell plate-integrated fluid selection device. If needed, we perforate side holes in the empty wells to communicate them with each other in order to use them as buffer wells (not shown).

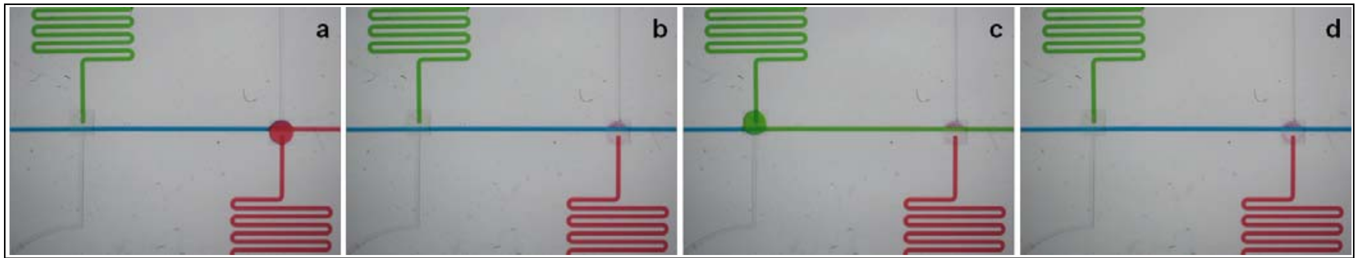

Figure 2: Micrographs showing the valve for green dye opened (a), followed by a rinse with buffer (b), the valve for red dye opened (c), and a second rinse with buffer (d).

The perfusion device is connected via a short tube to the perfusion chamber, which has an open roof (Figure 3). The floor of this perfusion chamber contains a PDMS microwell array for the cells. . After seeding, the chamber is reversibly sealed for perfusion with a coverslip and vacuum grease. The microwells (10 or 20 µm diameter, 10 µm deep) are arranged in sets of 5 x 5 blocks of 20 x 20 wells each, and each block is labeled with a unique set of coordinates (Figure 4).

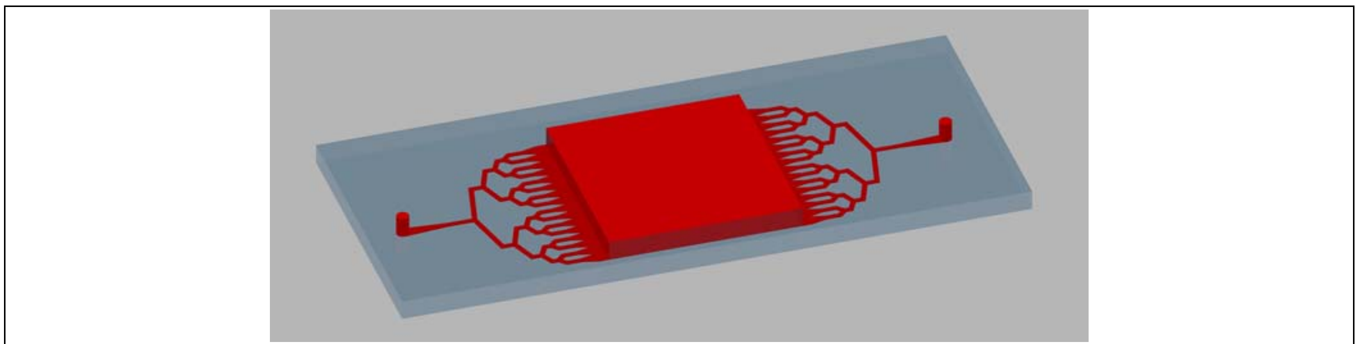

Figure 3: Schematic of the cell perfusion chamber with a removable roof.

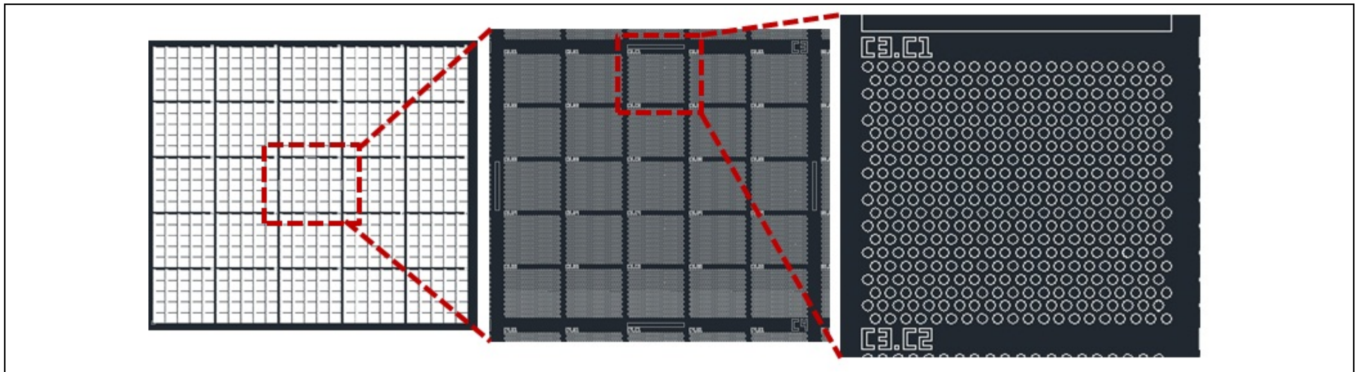

Figure 4: Mask design of the addressable microwell array.

EXPERIMENTAL

Arrays of 20 µm diameter microwells are oxygen plasma treated at 400 mTorr and 100 watts for 60 seconds. They are then coated with poly-D-lysine and concanavalin A type V for 90 minutes, followed by a rinse in deionized water. The olfactory epithelium lining the turbinates of two adult female C57/BL mice are dissected out and dissociated in papain. The OSNs are then loaded with the calcium-sensitive dye, fluo-4-AM, seeded onto the microwell substrate, and then incubated for 60 minutes to allow for dye loading and cell recovery. The cells are then sequentially perfused with Ringer's solution, DMSO control, a mix of 3 odorants (amyl acetate, benzyl acetate, heptanal, 100 µM each), farnesene (100 µM), and a high KCl solution. Cells responses are visualized as an increase in fluorescence of the fluo-4 with 4x images taken every 4 s.

RESULTS AND DISCUSSION

An example of acutely-dissociated OSNs responding to a non-specific depolarizing stimulus (KCl) and one OSN responding to the mouse pheromone, farnesene, are shown in Figure 5. In this example, there are many cells that respond to the application of KCl, indicating that those cells are neurons that are still viable at the end of our experiment. Smaller subsets of these cells responded to the application of the odorant mix and farnesene. We were able to find the locations of responsive cells (determined by changes in fluorescence intensity) by overlaying the fluorescence and phase contrast images of our microwell arrays. This demonstrates the potential to visualize and then stain and retrieve such rare cells using our addressable microwells.

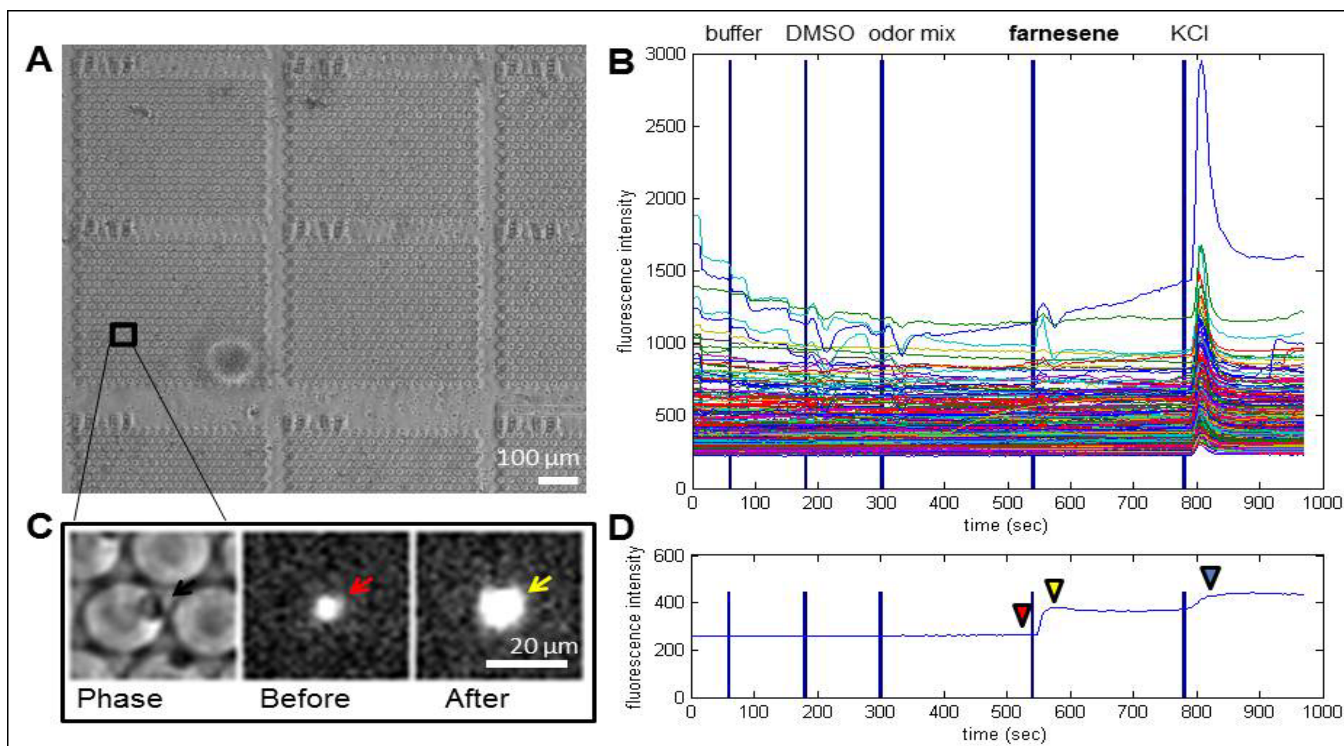

Figure 5: Micrographs (A,B) and response curves (C,D) of OSNs seeded onto the addressable microwells and then perfused with buffer, DMSO, a mix of 3 odorants, the pheromone farnesene, and a high KCl solution. The image in (A) represents ~8% of a 4x image. Panel (C) shows the responses of about ~400 KCl-responsive regions, and panels (B,D) depict the responses of a farnesene-responsive cell with responses to farnesene and KCl indicated with arrowheads.

CONCLUSION

We have developed an improved system for the trapping, perfusion, and imaging of olfactory sensory neurons. The integration of a 24-well microplate into our PDMS valving device simplifies the loading of fluids into our system by removing the need for externally-connected fluid reservoirs. Additionally, we are able to seed cells directly onto our addressable microwell arrays but still generate even flow profiles using our cell perfusion chamber with removable roof.

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