Electrically Active Microarray of 3D Carbon MEMS Electrodes for Pathogen Detection Systems

Jiae Shin
Department of Mechanical Engineering
San Diego State University

Abstract

The ability to isolate and concentrate pathogens (bacteria, virus, etc), bio-molecules and any sub-micron particle is critical to many biomedical applications, including diagnosis for cancer and infectious disease (SARS, deadly flu strains, STD, etc). Conventional two-dimensional active microarrays have been used with success for the manipulation of biomolecules including DNA. However they have a major drawback of inability to process relatively 'large-volume' samples useful in oncology and infectious disease applications due to its wash-away phenomena. This research presents an active microarray that exploits electrokinetic (electrophoresis and dielectrophoresis) forces for its hybridization method using 3D carbon electrodes that enables the large volume manipulation for pathogen detection. Carbon electrodes are fabricated using C-MEMS (Carbon MEMS) technology that is emerging as a very exciting research area nowadays. C-MEMS technology exploits excellent properties of carbon materials and also provides the advantage of low cost. The chip fabricated using C-MEMS technology is packaged and the efficiency of separation and accumulation of the 3D electrodes on the chip is tested by manipulating negatively charged polycarboxylate 2 μm beads in 50 mM histidine buffer.

Literature

Compared to many types of conventional pathogen detection methods such as culturing method, immuno-assay method, optical biosensor method etc, microarray-based pathogen detection method provides short assay time and massive screening capability by utilizing the benefits of microarrays. Microarrays are divided into two types, which are passive microarray and active microarray. Active microarray utilizes electrokinetic force for the hybridization of DNA molecules and it enables faster hybridization (in couple minutes) compared to passive microarray (at least 16 hours) where hybridization solely governs by diffusion. Conventional active microarrays that utilize two-dimensional electrodes have been used in success; however, it has a drawback of poor trapping due to its wash-away phenomena in the z-direction. As seen in Figure 1, the electric fields decays exponentially as the distance in the z-direction increases from the electrode [1]. Therefore, DNA molecules away from the electrodes experience no electric field and are washed away resulting in poor trapping.

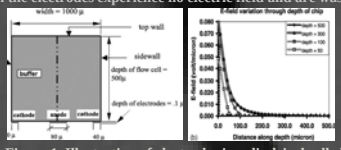


Figure 1. Illustration of electrodes in cylindrical cell chip and electric field variation through depth of chip
Courtesy of S. Kassegne *et al.*

However, three-dimensional (3D hereafter) electrodes provide not only increased surface area but also uniform electric field along the z-axis [2-5]. This enables better trapping in the z-direction and eventually achieves high-volume sample manipulation. This research utilizes 3D electrodes to improve trapping efficiency and the electrodes are microfabricated using Carbon MEMS (C-MEMS hereafter) technology which is an emerging fabrication method. In C-MEMS technology, structures are pre-patterned using conventional photolithography and later pyrolized to obtain conductive carbon material. Carbon-based materials exhibit unique physical, chemical, mechanical, and electrical properties: (i) compatible with inorganic and biological systems (ii) good thermal conductivity (iii) super-low friction (iv) chemical stability. C-MEMS technology utilizes these excellent properties of carbon-based materials to meet technological and economic needs [6].

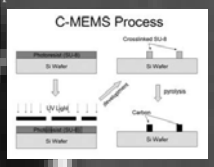
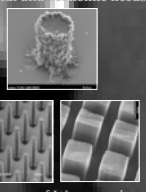


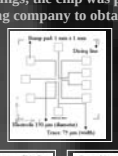
Figure 2. Schematic diagram of CMEMS process
Courtesy of M. Madou



Courtesy of Wang *et al.*

Microarray Design

First, the microarray design started with the simplest form which was 3 x 3 design that has 9 electrodes. Later, 5 x 5 and 10 x 10 microarrays were designed to increase the work-efficiency. As the number of test sites increases, it became more difficult to achieve efficient traces layout that connects the electrodes with bump pads as seen in Figure 3. By changing the shape of electrodes from circular shapes to diamond shapes, three more designs were achieved. Both the diameter of each circular-shaped electrode and the width of diamond-shaped electrode are 150 μm and the spacing between center to center of each electrode is 350 μm. The width of traces that connect the electrodes and the bump pads is 75 μm. The bump pad size is 1 mm x 1 mm and the overall die size is 1 cm x 1 cm. Using six desings, the chip was populated as seen in Figure 4 and the populated chip CAD files were sent to the printing company to obtain masks that is used in the microfabrication process (for lithography).



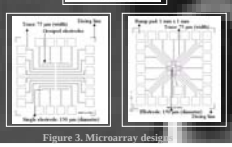
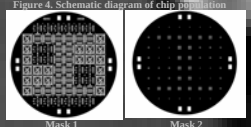


Figure 3. Microarray design

A1 – 3x3 – 2 – 3x3D
B1 – 5x5 – 2 – 5x5D
C1 – 10x10 – 2 – 10x10D
10 dies of each design.
Tot = 60.

Figure 4. Schematic diagram of chip population



Mask 1 Mask 2

Figure 4. Mask 1 for electrical connections and mask 2 for electrode posts

Reference

[1] K. Y. Lu, A. M. Wo, Y. J. Lo, K. C. Chen, C. M. Lin, and C. H. Yang. "Three-dimensional elctrode array for cell lysis via electroporation." Biosensors and Bioelectronics, Vol. 22 (2006): 568-574.
[2] N. Honda, M. Inaba, T. Katagiri, S. Shoji, H. Sato, T. Hosokawa, T. Osaka, M. Suzuki, J. Mizuno, and Y. Wada. "High Efficiency Electrochemical Immuno Sensors Using 3D comb Electrodes." Biosensors and Bioelectronics, Vol. 20 (2005): 2306-2309.
[3] F. E. H. Tay, L. Yu, A. J. Pang, and C. Iliescu. "Electrical and thermal characterization of a dielectrophoretic chip with 3D electrdes for cells manipulation." Electrochimica Acta, Vol. 52 (2007): 2862-2868.
[4] C. Iliescu, L. Yu, G. Xu, and F. E. Tay. "A Dielectrophoretic Chip with a 3D Electric Field Gradient." Journal of Microelectromechanical Systems, Vol. 15, No. 6 (2006): 1506 - 1513
[5] L. Yu, C. Iliescu, G. Xu, and F. E. Tay. "Sequential Field-Flow Cell Seperation Method in a Dielectrophoretic Chip with 3D Electrodes." Journal of Microelectromechanical Systems, Vol. 16, No. 5 (2007): 1120-1129.
[6] S. Maldonado and K. J. Stevenson. "Direct Assembly of 3D Carbon Nanofiber Electrodes for Electrochemical Power Sources." Abs. 1286, 204th Meeting , © 2003 The Electrochemical Society, Inc.

Microfabrication

Microfabrication of 3D C-MEMS electrodes microarray consists of three big steps that are first lithography, second lithography, and pyrolysis. The first lithography is carried out to achieve electrical connections between electrodes and bump pads and the second lithography is for electrode post. Lastly, by pyrolyzing the pre-patterned structures, conductive carbon materials are obtained. C-MEMS microfabrication processes are shown in the Table 1.

Table 1. C-MEMS microfabrication process

Process	Equipment	Chemical used	Thickness	Note
Clean wafer		Acetone/ methanol		
Grow oxide	Oven		0.5 μm	
First lithography				
Spin-coating	Spinner	SU-8 10	10 μm	35 sec. @ 3000 rpm
Soft-bake	Oven			7 min. @ 95ºC
UV exposure	UV exposure machine			Use mask 1/ with the energy of 300 mJ/cm2
PEB				4 min. @ 95ºC
(PostExposueBake)	Leveled hot plate			
Develop	Wet bench	SU-8 developer/ Isopropanol		
Second lithography				
Spin-coating	Spinner	SU-8 100	220 μm	35 sec. @ 1600 rpm
Safe-bake	Oven			1.5 h @ 95ºC
Alignment & UV exposure	Mask aligner			Use Mask2/ with the energy of 700 mJ/cm2
PEB	Leveled hot plate			20 min. @ 95ºC
Develop	Wet bench	SU-8 developer/ Isopropanol		30 min.
Pyrolysis	Furnace	Nitrogen gas		Pyrolyze according to Heating schedule

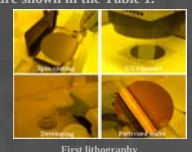


Figure 5. Microfabrication process

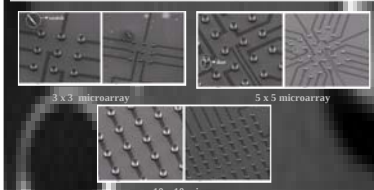


Figure 6. SEM images of each design after pyrolysis

Experimentation

Experimentation was conducted using negatively charged 2 μm polycarboxlate microbeads purchased from Polysciences co. in 50 mM histidine buffer in both open-channel electrophoresis and closed-channel electrophoresis on the probe station.

In open-channel electrophoresis the chip was mounted on the open-channel mount and the needles from probe arms directly touched the bump pad for the electrical access as seen in the Figure 7. Beads accumulation is clearly seen in Figure 8.

Figure 7. Open-channel electrophoresis set up

Figure 8. Beads accumulation in open-channel electrophoresis

In closed-channel electrophoresis, packaging was used for the isolation of electrode sites from the bump pads to prevent the accumulation at the bump pads. Packaging set consists of three parts, which are top case that has PDMS (Polydimethylsiloxane) pressure seal inside at the bottom, PDMS pressure seal, and bottom case that has chip mount site as seen in Figure 9. For the electrical access, bump pads were grouped together using conductive silver paste and the needles from probe arms touched the silver paste through bump pads windows as seen in Figure 10.

Figure 9. Packaging for closed-channel electrophoresis experimentation

Figure 10. Closed-channel electrophoresis set up

3 x 3 microarray 10 x 10 microarray

Figure 11. Beads accumulation in closed-channel electrophoresis

In both open-channel and closed-channel electrophoresis, beads accumulation was observed at 4-5 V. Compared to conventional 2D active microarray, 3D C-MEMS active microarray utilized in this research provides wider electrochemical window (Figure 12.) which is directly related to accumulation speed since velocity of DNA molecules is proportional to electric field (velocity= mobility x electric field). Also FEA simulations was carried out using Femlab Comsol 3.3 and the results qualitatively matched with experimental results as seen in Figure 13.

Figure 12. Electrochemical window comparison

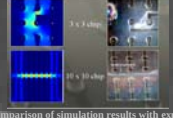


Figure 13. Comparison of simulation results with experimental results

Conclusion

In this research, high-efficiency and high-volume sample manipulation active microarray was achieved by utilizing 3D electrodes. Unlike the 3D electrodes reported in earlier researches, 3D electrodes utilized in this research were fabricated using C-MEMS technology that exploited the excellent properties of carbon-based materials. Also, the uniqueness of 3D C-MEMS electrodes microarray is that it provides wider electrochemical window that is related to the accumulation speed compared to conventional 2D electrodes microarray.

Acknowledgement

I'd like to thank my advisor, Dr. Samuel Kassegne who guided and encouraged me throughout this research. Also I'm thankful for Genis at UCI who contributed to C-MEMS microfabrication process and 585 MEMS project group members who helped me with some packaging and experimentation.