Point-of-Care (POC) Micro Biochip for Cancer Diagnostics

Bharath Babu Nunna* and Eon Soo Lee *

*New Jersey Institute of Technology, NJIT
200 Central Ave, Newark, New Jersey, USA, eonsoo.lee@njit.edu

ABSTRACT

Most of the cancers are curable if they are detected at early stages. The early stage detection of cancers can significantly improve the patient treatment outcomes and thus helps to decrease the. To achieve the early detection of the specific cancer, the biochip is incorporated with an innovative sensing mechanism and surface treated microchannels. The sensing mechanism employed in the Point of Care (POC) biochip is designed to be highly specific and sensitive. The surface treated microchannel helps to control the self-driven flow of the blood sample. Cancer antibodies with enhanced specificity and affinity are specially developed and immobilized on the surface of the nano circuit in microchannel. When the blood sample flows in the microchannel over the cancer antibodies, the corresponding cancer antigens from the blood forms the antigen-antibody complex. These antigen-antibody interactions are captured with the variation in the electrical properites of the gold nano circuit using the sening mechanism in the biochip. The point of care (POC) micro biochip is designed as an in-situ standalone device to diagnose the complex cancers like ovarian cancer at the early stages by sensing the cancer antigens in the blood sample with very low concentrations (to the level of femto scale) from the blood sample drawn from a finger prick. The POC biochip can help to diagnose, the existence of cancer and also its severity using the qualitative and the quantitative results of the sensing mehanism in the biochip. The intial experimental results of the POC biochip detected the cancer antigens (CA-125) at the nano level concentrations in the sample.

Keywords: biochip, point-of-care (POC), self-driven flow, cancer diagnosis, nano circuit with capacitive sensing

1 INTRODUCTION

The American Cancer Society stated that a total of 1,688,780 new cancer cases and 600,920 deaths from cancer are projected to occur in the United States in 2017 [1]. Cancer still remains the second most common cause for death in the United States, which is about 1 in every 4 deaths. For example, ovarian cancer ranks as the fifth most common cancer in women and also has the highest mortality rate among all the gynecologic malignancies. The current technologies available detect only 15% of ovarian cancer cases at early stages (ie., stage 1A & stage 1B), with the survival rate of 93%, while the remaining 85% of ovarian cancer cases are detected at the advanced stages, with the survival rate being just 31%. The significant rise of the survival rate of these cancer cases, emphasises the need of early stage diagnosis. Enabling the cancer diagnosis as an easy and simple process can increase the number of diagnosis and thus have an increased chances of early stage diagnosis.

Cancer diagnostics is still a frontier that has not been completely explored by biochip researchers. In this research, the POC micro biochip (Fig-1) is primarily intended to diagnose complex cancers like ovarian cancer at the early stage with a blood sample from a finger prick.

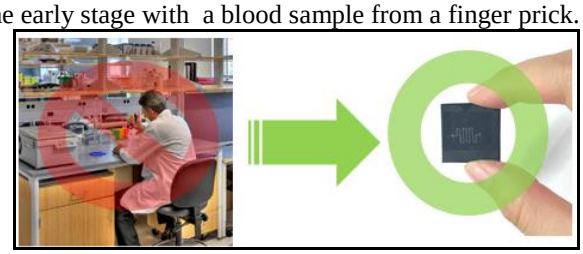

Fig-1. The conventional cancer diagnosis process (Left) & POC micro biochip model (Right).

2 BIOCHIP DESIGN

The POC micro biochip is incorporated with surface treated microchannels to control the self-driven flow of biofluids without any external flow control devices and a capacitance sensing mechanism to detect the biological interactions such as antigen (Ag)-antibody (Ab) complex formation (Fig-2). The biochip is designed with multichannel distribution from a single inlet for the blood sample, to improve the feasibility of detecting multiple antigens from the same sample of blood simultaneously with enhnaced specificity. In POC biochip the multiple gold nano Interdigitated Electrodes (IDE) are incorporated at different sections of the microchannel to sense the biological interactions with the enhnaced signal and thus increase the sensitivity. The gold nano IDEs are connected to individual contact pads, to monitor the signal from each IDE separately. A specific antibody that can interact the the targeted antigen is immobilized on the surface of the nano circuit. Attaching each IDE with an unique cancer antibody helps to detect the concentration of corresponding antigen individually, by sensing the signal of antigen-antibody complex formation at each IDE individually. The existence of cancers can be determined from the blood sample, by detecting the corresponding cancer antigens. Thus the POC biochip is designed to detect the multiple cancers with enhnaced sensitivity and specificity [2-4].

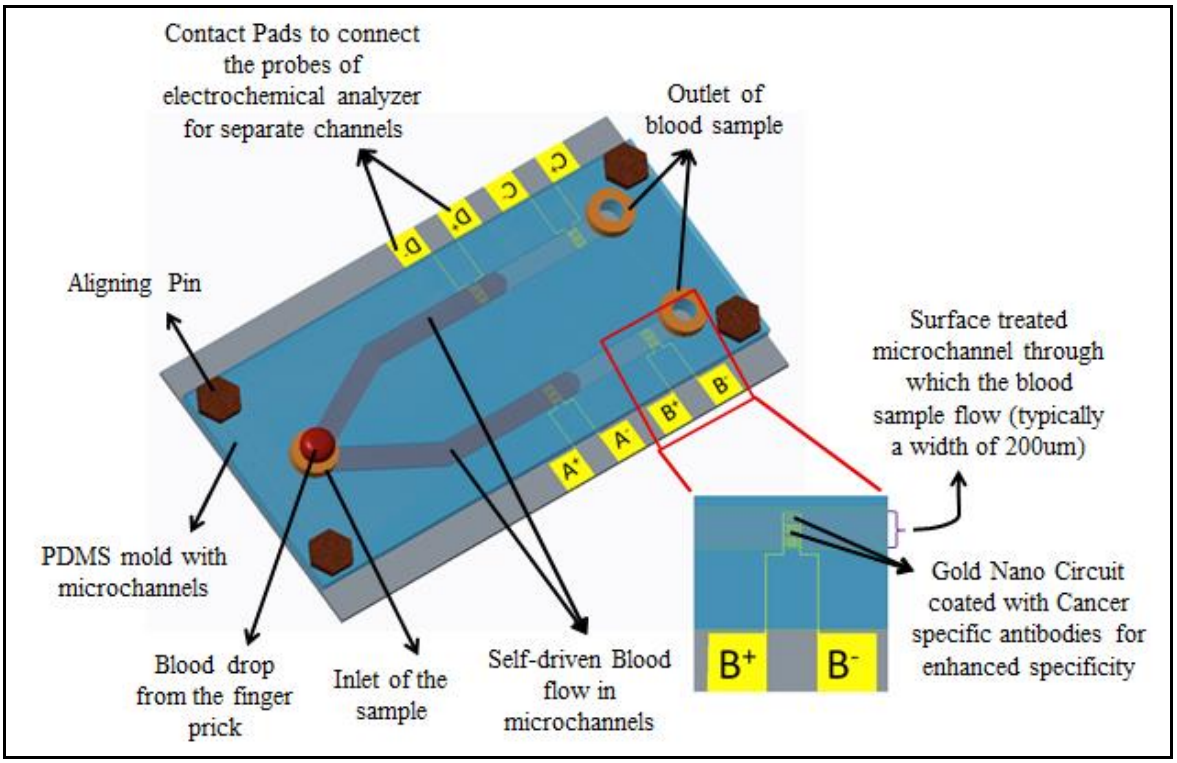

Fig-2: Schematic model of POC micro biochip with microchannels and gold nano circuit

2.1 Surface treated microchannels

The microchannels are designed with the with specific aspect ratios to amplify the self-driven capillary flow and self-separation of biomolecules from biofluid. A Si wafer of 4 inch diameter is cleaned and spin coated with a negative photoresist (SPRTM 955), which is then exposed to UV rays using the UV mask aligner for 14 seconds. The wafer is then treated with CD-26 and DI water to let the photoresist remain only at the microchannel structures. The Si wafer is then etched using Deep Reactive Ion Etching (DRIE) to 107um, which elevate the microchannel structures and remove the material from rest of the areas. Polydimethylsiloxane (PDMS) is mixed with appropriate composition (1:10) and then poured on top of etched Si-wafer with microchannel structures after degassing at vaccum chamber. The PDMS along with the Siwafer is baked at 60 degree centigrade for an hour. Then the PDMS layer is carefully peeled and made holes for the inlet and oulet to the microchannel as shown in the Fig-3. The PDMS mold is then aligned with Si-wafer with nano circuit.

PDMS is highly inert and hydrophobic in its nature. To convert the PDMS to hydrophilic, the PDMS surface is exposed to oxygen plasma for various durations. In this experiment, the hydrophilicity of PDMS controlled by the variation in duration of the plasma treatment. The plasma treatments are performed on the 'Plasma Cleaner- PDC-32G' with oxygen flow rate of 20sccm and 98.8 bar pressure. The radio frequency (RF power supply-150W) of 13.56 MHz frequency is used for plasma excitation.

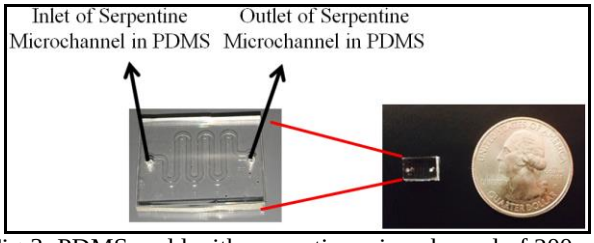

Fig-3: PDMS mold with serpentine microchannel of 200um width and 107 um height with U.S quarter coin for size comparision

2.2 Gold nano interdigitated circuit

To fabricate the gold nano circuit on the Si wafer, initially it is cleaned with isopropanol and coated with a positive photoresist (PMMA-A6). Considering the required height of the PMMA, the spin coater is set to appropriate speedand later it is dried before it follows the lithograpgy steps at the Electron Beam Lithography (EBL) tool. The desired nano circuit pattern is formed on the Si wafer after it is washed with MIBK:IPAf for 60 seconds. To improve the adhesion between gold and silicon, a layer of Titanium (app 10nm) is deposited. 90nm of gold is deposited on the nano circuit patterned Si wafer, using the Physical Vapor Deposition (PVD) process using Kurt J Lesker PVD-75 Evaporator. The lift-off process is implemented to remove the photoresist by cleaning with Acetone ultrasonic bath and later dried with nitrogen gas.

The gold nano electrodes are insulated with the Self-assembled monolayer (SAM) and then coated with cancer antibodies. To form the SAM layer The electrodes are immersed in a 50mM Thiourea solution for 12 hours (Fig-

4). Then the surface of the electrodes are rinsed with ethanol and Millipore deionized water and dried using Nitrogen gas. Glutaraldehyde is used to promote surface activation on the SAM layer. The CA-125 antibodies are aliquotted with a concentration of 10 ng/ml and then placed on top of the surface activated SAM layer at 4°C for 12 hours to immobilize the antibodies. To block the unwanted sites or the bare spots on electrode surface A 10mM of 1-dodecanthiol in ethanolic solution was added on top (Fig-5). The PDMS microchannel is properly aligned with the nano patterned interdigitated circuit to facilitate the blood sample to flow on the cancer antibodies.

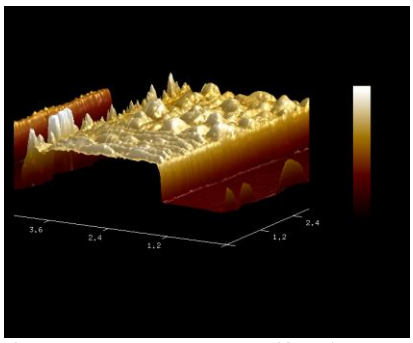

Fig-4: The Atomic Force Microscopic (AFM) image of the gold interdigitated electrodes with SAM layer.

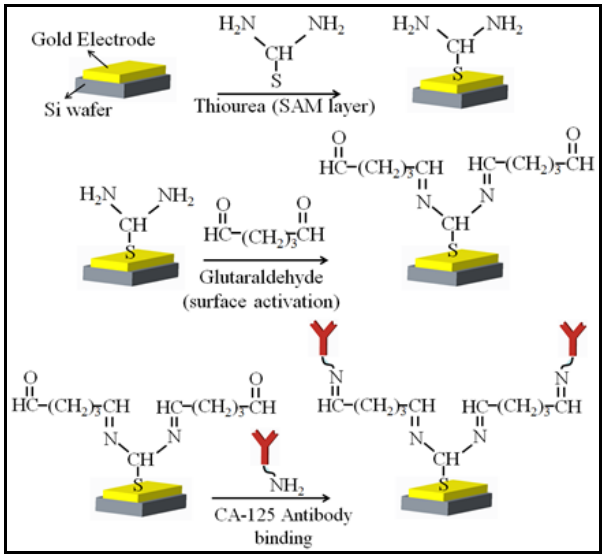

Fig-5: Schematic representation of CA-125 Cancer antibody immobilization on nano gold interdigiated electrodes.

3 RESULTS AND DISCUSSION

3.1 Controlled self-driven flow of blood in microchannel

As per Thomas Young, the contact angle of the liquid drop on the solid surface is defined by the mechanical equilibrium of the drop, with the influence of the interfacial tensions. The three interfacial tensions identified when a blood drop is placed on a solid (PDMS) surface are $\gamma_{blood,air}$, $\gamma_{soild,air}$ & $\gamma_{blood,solid}$, where $\gamma_{blood,air}$ is the interfacial tension between the blood and air, $\gamma_{soild,air}$ is the interfacial tension between the PDMS substrate and air, and $\gamma_{blood,solid}$ is the interfacial tension between blood and PDMS substrate.
As per Young's law,

$$\gamma_{solid,air} = \gamma_{blood,solid} + \gamma_{blood,air} \cos\theta \quad (1)$$

From eq (1), the contact angle θ can be calculated, as per eq (2),

$$\text{Cos}\theta = \left(\frac{\gamma_{solid,air} - \gamma_{blood,solid}}{\gamma_{blood,air}} \right) \quad (2)$$

The surface tension is the primary cause of the capillary pressure difference across the interface between two fluids (liquid and air).

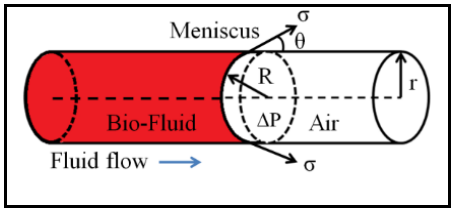

Fig-6: Schematic of the biofluid flowing in capillary channel due to surface tension.

The schematic of the microchannel (Fig-6), of circular cross section with radius r, that is filled with two immiscible fluids (Biofluid and air) with surface tension σ, the meniscus is approximated as a portion of a sphere with radius R, and the pressure difference across the meniscus is:

$$\Delta P = -\frac{2\sigma}{R} \quad (3)$$

The radius R of the meniscus depends only on the contact angle θ and the radius of the channel r as in eq-4:

$$\Delta P = -\frac{2\sigma \text{Cos}\theta}{R} \quad (4)$$

By varying the contact angle of the fluid with the necessary surface treatments to the surface of microchannel helps in controlling the self-driven flow, when driven by the surface tension.

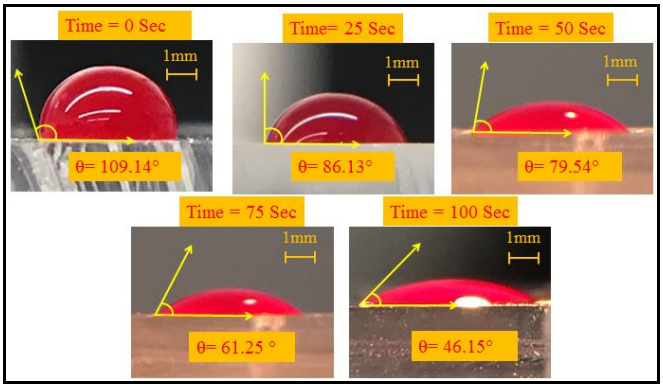

Fig-7. Blood Drop Images (4.2ul volume) on PDMS surface treated with oxygen plasma for various durations.

The surface treatment on the PDMS surface helps in controlling the contact angle from a range of 109.14° to 46.15°(Fig-7). Increasing the duration of oxygen plasma treatment to PDMS surface has decreased the contact angle of the blood drop on the PDMS surface. This explains that the PDMS surface is converted from hydrophobic to hydrophilic nature with the duration of surface treatment (Fig-8).

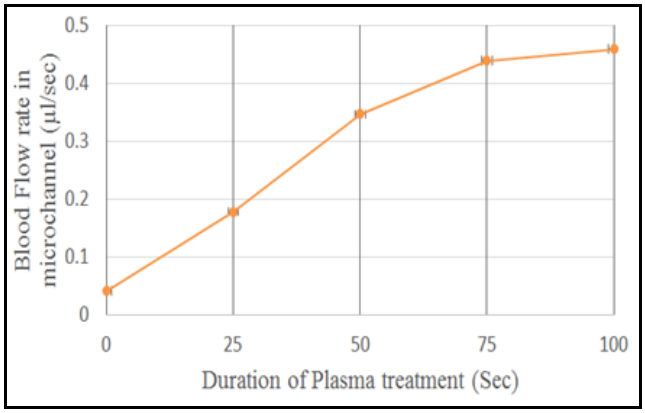

Fig-8: Plot of the flow rate variation with the surface treatment duration

3.2 Sensing Cancer antigen CA-125 from the blood sample

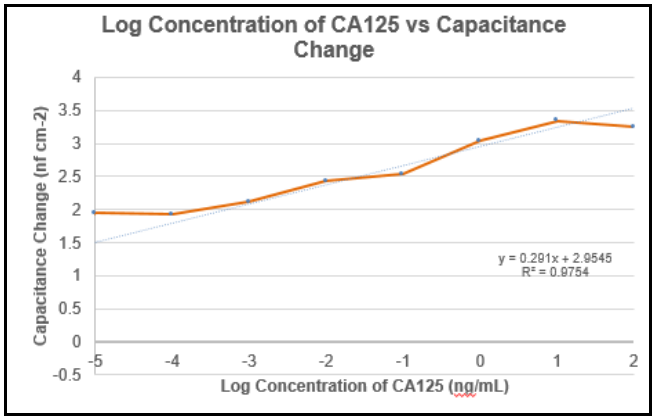

Fig-9: Plot of Capacitance Variation vs the logarithm of antigen concentration of CA-125

The above plot explains the log concentration variation in the analyte, will generates the change in the capacitance at nano level as shown in Fig-9. The experimental results provide the evidence of change in capacitance with the change in the analyte due to the antigen and antibody interaction. The nano scale capacitance variation is detected in POC micro biochip due to antigen/antibody complex formation with the cancer antigens CA-125 in the sample with nano scale concentration. The capacitance change is caused due to the formation of antigen and antibody complex formation in the microchannel. Detecting the existence of Cancer antigens (CA-125) from the blood sample helps to determine the cancer.

4 CONCLUSION

The self-driven flow in the microchannel is controlled by the surface treatments on the microchannel surface. The controlled flow rate in microchannels provides necessary conditions for biological reactions like antigen- antibody complex formation. Controlling the flow rate without any external devices helps to minimize the contamination of the sample. The change in the capacitance due to the nano level concentration of the cancer (CA-125) antigens in the the blood sample helps to determine the existence of cancer. The biochip research is currently progressing to detect the ovrian cancer antigens using biomarkers like anti-peptide antibodies which are raised against Kalikrein-6 (KLK6), Kalikrein-7 (KLK7) and Protease Serine-8/ Prostasin (PRSS8), in the concentrations of pico and femto levels with the enhnaced sensing mechanism. The information of existense of cancer antigens enable the physicians to schedule the patient for next level of cancer diagnosis. This research work promotes in developing new standalone POC devices with non-optical sensing mechanisms.

5 ACKNOWLEDGEMENTS

The authors acknowledge the research support from New Jersey Institute of Technology (NJIT). This research is carried out in part at the Center for Functional Nanomaterials, Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, under Contract No. DE-SC0012704. Also this research is supported by John Theurer Cancer Center in Hackensack University Medical Center at Hackensack, New Jersey.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2017. Atlanta; American Cancer Society; 2017
2. Nunna, B.B., Zhuang, S., Javier, J., Mandal, D., Lee, E.S. 2016, November. Biomolecular Detection using Molecularly Imprinted Polymers (MIPs) at Point-of-Care (POC) Micro Biochip. In IEEE-NIH Healthcare Innovation Point-Of-Care Technologies Conference.
3. Nunna, B.B., Zhuang, S., Malave, I., Lee, E.S. 2015, November. Ovarian Cancer Diagnosis using Micro Biochip. In NIH-IEEE Strategic Conference on Healthcare Innovations and Point-of-Care Technologies for Precision Medicine Conference.
4. Zhuang, Shiqiang, Eon Soo Lee, Lin Lei, Bharath Babu Nunna, Liyuan Kuang, and Wen Zhang. "Synthesis of nitrogen doped graphene catalyst by high energy wet ball milling for electrochemical systems." International Journal of Energy Research 40, no. 15 (2016): 2136-2149.