ELECTROCHEMICAL DETECTION OF CANCER CELLS ON A CENTRIFUGAL MICROFLUIDIC PLATFORM

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ABSTRACT
This paper reports for the first time the electrochemical detection of MCF7 breast cancer cells on our unique dissolvable film (DF) – based centrifugal platform through the integration of ~50-nm thick gold electrodes on a plastic disc substrate. On this platform, we demonstrate an integrated system that is amenable for blood separation, extraction, capture and electrochemical detection of the MCF7 breast cancer cells on the surface of EPCAM-antibody functionalized gold microelectrodes by mere impedance measurements.

KEYWORDS
Electrochemical detection, microfluidics, centrifugal, cancer, MCF7

INTRODUCTION
In comparison with optical detection techniques, biological electrochemical impedance spectroscopy (bio-EIS) has attracted great attention in the recent past, as it is a direct non-destructive, label-free and highly sensitive method which involves low-complexity instrumentation. Significantly, with this technique, electrochemical detection of analytes is enabled with minimum or even without sample preparation. Also complete integration into lab-on-a-chip systems for point of care diagnosis has been established.[1, 2] In this paper, we report for the first time on a much simpler integration of electrochemical sensors on a centrifugal platform which has proven to allow high-performance cell handling and analysis.[3-5]

ASSAY INTEGRATION AND ELECTROCHEMICAL DETECTION
We have recently demonstrated the integration of a multi-step immunoassay (IA) protocol on a centrifugal "lab-on-a-disc" platform using our novel DF valves.[6] A schematic illustration of the disc is shown in Fig. 1A. Figure 1B demonstrates the fluidic, multi-step assay protocol. Prior to the start of the assay procedure, whole blood spiked with cancer cells is pre-loaded in the sedimentation chamber, BSA block solution and PBS wash buffers are introduced into their respective storage chambers. The working principle of the DF-based valving technique is illustrated in Fig. 1C.

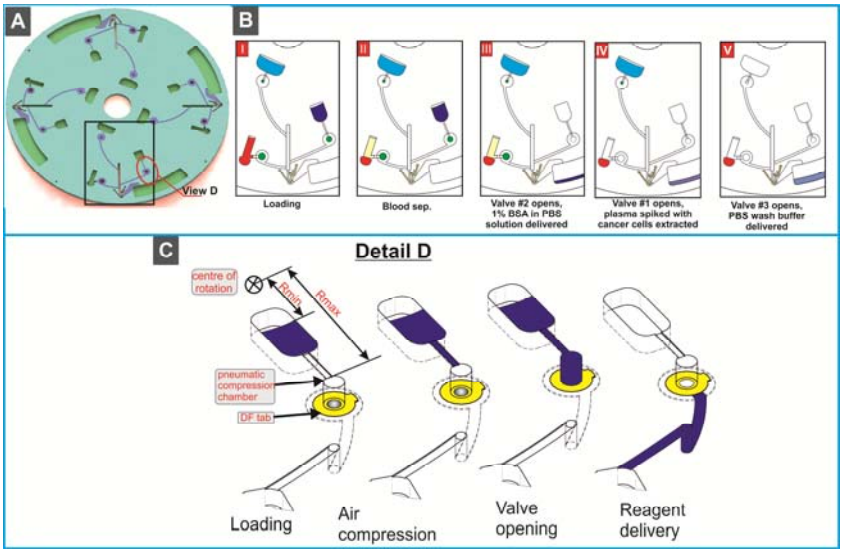

Figure 1 a) 3D illustration of the DF-based centrifugal platform b) schematic illustration of the multi-step IA c) Step-by-step demonstration of the DF valve actuation by rotation of the disc.

Briefly, the DF valves are only rotationally actuated by trapping an air pocket above a pneumatic chamber. During rotation, a critical burst pressure is reached, at which the metastable air-liquid layer collapses, and liquid makes contact and dissolves the DF, thus clearing the passage for the liquid (Fig. 1C). The assembly of the 5-layer disc is demonstrated in Fig. 2A. The disc is made from 2 layers of pressure sensitive adhesives sandwiched between 3 layers of 1.5-mm thick PMMA plastic substrates.

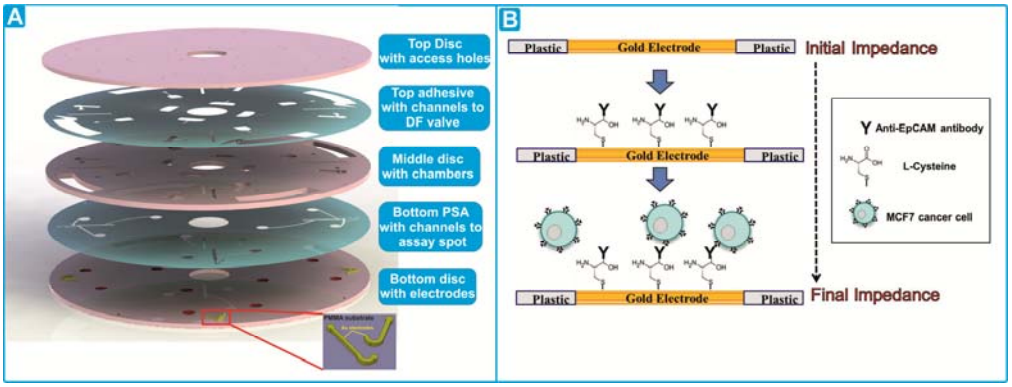

Figure 2: a) 5-layer disc assembly. 2 adhesive layers are interspersed between 3 plastic substrates. b) Schematic illustration of the surface chemistry, Ab immobilization and cell capture. Initial impedance measurements are carried out before Ab immobilization and after cell capture.

Approximately 50 nm of gold is patterned on the bottom disc by sputtering technique. 50 µL of 100 µg/mL anti-EpCAM antibody (Ab) is immobilized on the gold electrodes prior to disc assembly. Figure 2B shows a schematic of the antibody immobilization on gold electrodes, MCF7 cancer cell capture and electrochemical detection using impedance measurements.

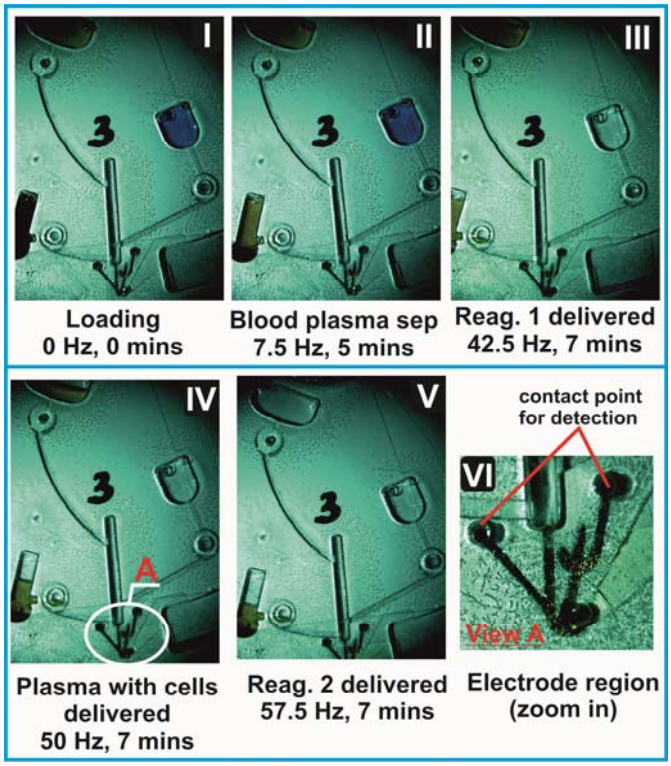

Figure 3: I-V) Frame sequence of the multi-step IA showing blood separation and sequential reagent release. In order to enhance contrast, 1% v/v food dye was added to PBS buffer, and pre-loaded in the reagent chambers. VI) View of the electrode region and contact point for electrochemical detection.

Figure 3 shows the frame sequence of the multi-step IA including blood separation and extraction of cells along with the detailed spinning protocol from sample loading to detection. At present, immobilization of Ab, capture and detection of cancer cells on the gold electrodes are carried out off-chip. The antibody coated gold electrodes were studied using cyclic voltammetry (Fig. 4a) and impedance spectroscopy (Fig. 4b) and were confirmed by fluorescence imaging (Figs. 4c-d). It can be clearly seen that due to the presence of specifically captured cancer cells, the diffusion of the FeOH redox probe to the surface of the electrode is hindered and the current drops from 2.1 µA to 1.78 µA. Impedance measurements (Fig. 4b) show that there is also a significant increase in the admittance on cell capture.

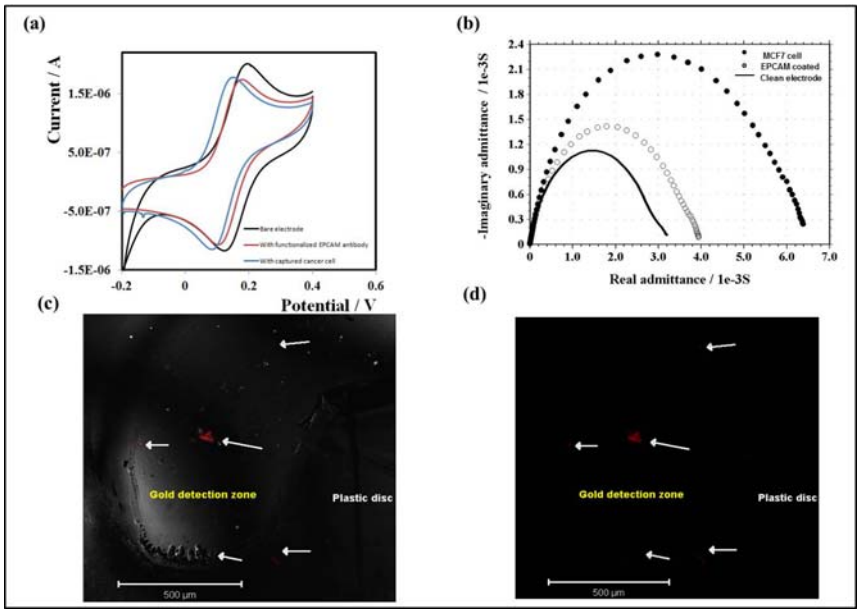

Figure 4: a) Cyclic voltammetry showing the different curves for the bare electrode, with Ab and with cells b) Corresponding EIS measurements c-d) fluorescence images of the captured cells on the Au electrodes. Capture and detection of the cancer cells was carried out off-chip.

CONCLUSION & OUTLOOK

We have demonstrated for the first time electrochemical detection of MCF7 breast cancer cells on our novel, rotationally actuated DF-based centrifugal platform. This novel point-of-care platform is capable of carrying out a multi-step immunoassay starting from whole blood for the specific capture and sensitive label free detection of cancer cells.

In the future we plan to further integrate the platform towards a full-fledged sample-to-answer device.

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