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Label-free impedance detection of cancer cells from whole blood on an integrated centrifugal microfluidic platform

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ABSTRACT

An electrochemical Lab-on-a-Disc (eLoaD) platform for the automated quantification of ovarian cancer cells (SKOV3) from whole blood is reported. This centrifugal microfluidic system combines complex sample handling, i.e., blood separation and cancer cell extraction from plasma, with specific capture and sensitive detection using label-free electrochemical impedance. Flow control is facilitated using rotationally actuated valving strategies including siphoning, capillary and centrifugo-pneumatic dissolvable-film (DF) valves. For the detection systems, the thiol-containing amino acid, L-Cysteine, was self-assembled onto smooth gold electrodes and functionalized with anti-EpCAM. By adjusting the concentration of buffer electrolyte, the thickness of the electrical double layer was extended so the interfacial electric field interacts with the bound cells. Significant impedance changes were recorded at 117.2 Hz and 46.5 Hz upon cell capture. Applying AC amplitude of 50 mV at 117.2 Hz and open circuit potential, a minimum of 214 captured cells/mm^2 and 87% capture efficiency could be recorded. The eLoaD platform can perform five different assays in parallel with linear dynamic range between 16,400 and $(2.6 \pm 0.0003) \times 10^6$ cancer cells/mL of blood, i.e. covering nearly three orders of magnitude. Using the electrode area of 15.3 mm^2 and an SKOV3 cell radius of 5 μm, the lower detection limit is equivalent to a fractional surface coverage of approximately 2%, thus making eLoaD a highly sensitive and efficient prognostic tool that can be developed for clinical settings where ease of handling and minimal sample preparation are paramount.

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1. Introduction

Metastasis is the main cause of cancer-related deaths worldwide and it is estimated to rise over 20 million in 2030 according to World Health Organization (Jemal et al., 2011). Progression of the disease is the result of a multistage process during which circulating tumour cells (CTCs) typically detach from the primary tumour site and circulate in the blood stream. These CTCs tend to be very rare (Sheng et al., 2012); it is often assumed their concentration may be as low as ~1–100 cells/mL in whole blood (Coumans et al., 2013; Hou et al., 2013). Nevertheless, their concentration in blood has (Pantel et al., 2008) diagnostic and prognostic clinical relevance (Dotan et al., 2009; Lianidou et al., 2012; Scher et al., 2009; Wicha and Hayes, 2011). Current methods for the detection and staging of cancer are mostly pathological and provide clinicians with a snapshot of the primary tumour and the spread of cancer from one organ to another, but not necessarily the total tumour burden. This deficit makes it challenging to accurately predict patient outcome or directly guide treatment. Also, chiefly owing to their extremely low concentration, the isolation and detection of CTCs within the human bloodstream make their reliable isolation and detection a highly complex and time consuming task involving expensive instrumentation, resources and highly trained laboratory staff. Capture of (viable) CTCs using inexpensive rapid diagnostics may offer a new way to detect metastasis and prognose patients as well as providing molecular level insights into the genetic profile of the cancer that will guide the selection of the optimum treatment.

CTCs are inherently variable with respect to their density, size, size distribution, morphology, deformability and cell surface composition (Jin et al., 2014), thus demanding a highly flexible capture and detection system. Consequently, techniques including density-gradient based centrifugation (Park et al., 2012), micro-

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filtration (Zheng et al., 2011), hydrodynamic sorting by size (Loutherback et al., 2012), separation by cell deformability (Tan et al., 2009), immunoassay based on tumour-specific surface protein expression (Alix-Panabieres and Pantel, 2014), and molecular markers (Nagrath et al., 2007) have been employed to effectively separate these cells from normal blood cells before capture. Subsequent enumeration of captured CTCs has been demonstrated with such techniques as immunofluorescence (Ignatiadis et al., 2008), confocal microscopy (Greiner et al., 2011), absorbance (Vila-Planas et al., 2011), chemiluminescence (Hun et al., 2010), interference spectroscopy (Kumeria et al., 2012), surface-enhanced Raman scattering (Lee et al., 2014b) and surface plasmon resonance (Law et al., 2011). However, a simple, sensitive and robust assay that allows routine detection and subsequent molecular characterization of CTCs in a clinical setting has not been reported.

Centrifugal platforms for bioanalytical assays have been investigated for more than 40 years (Ducrée et al., 2007; Gorkin et al., 2010; Madou et al., 2006). For cell separation, these Lab-on-a-Disc (LoaD) platforms excel with respect to more common pressure-driven systems due to the simplified operation without pneumatic interfaces and pumps, the capability for powerful sample preparation and the demonstrated amenability for full integration, automation and parallelization of comprehensive bioanalytical assay protocols (Duford et al., 2013; Focke et al., 2010; Gorkin et al., 2012; Kim et al., 2014; C. Nwankire et al., 2013; Nwankire et al., 2014; C.E. Nwankire et al., 2013). LoaD platforms have also been developed for cell handling, bioassays (Boettcher et al., 2006; Burger and Ducrée, 2012; Burger et al., 2012; Chen et al., 2012; Lee et al., 2014a) and have also been interfaced with electrical systems for real-time flow monitoring (Abi-Samra et al., 2013). For cell detection, electrochemical schemes are beneficial due to their ability to provide highly sensitive, rapid real-time quantitative information, based on simple instrumentation. When combined with electrochemical impedance, electrochemical detection can also be label-free and non-destructive (Venkatanarayanan et al., 2013).

Hence, combining the benefits of electrochemical impedance spectroscopy (EIS) and LoaD platforms, we here demonstrate for the first time, label-free electrochemical detection of ovarian cancer cells in a fully integrated, multiplexed, automated eLoaD platform.

2. Experimental section

2.1. Chemicals and solutions

Sulphuric acid, sulpho-N-hydroxy succinimide (NHS), 1-ethyl-3-[3-dimethyl-amino-propyl] carbodiimide hydrochloride (EDC), 2.5% casein, paraformaldehyde and fetal bovine serum (FBS), L-Cysteine hydrochloride, ethylene diamine tetraacetic acid (EDTA), Ficoll PM 400 were purchased from Sigma-Aldrich and used as received. The SKOV3 ovarian cancer cell line was purchased from ATCC and stored in liquid nitrogen. Details of the cell culture protocol are given in S1-ESI. Purified anti-EpCAM (CD326) antibodies were obtained from Biolegend. McCoy's 5A (containing L-glutamine), penicillin streptomycin (pen/strep), Dulbecco's phosphate buffered saline (DPBS) without calcium and magnesium, as well as trypan blue were all purchased from Fisher Scientific. Accutase cell dissociation buffer was bought from Invitrogen. Experiments involving cells were carried out in a certified Biosafety Level II laboratory under sterile, laminar air flow conditions. Cells were fixed following impedance measurements with 3.5% (w/v) paraformaldehyde. All aqueous solutions were prepared using milli-Q water (18 $M\Omega\ cm^{-1}$).

2.2. eLoaD fabrication

The eLoaD platform is made up of five layers in total – two adhesive layers were sandwiched between three PMMA polymer layers (Fig. 1A). Computer-Aided-Design (CAD) files of the vents, vias and reservoirs were machined in the 1.5-mm thick PMMA (Radionics, Ireland) layers using a CO_2 laser cutter (Zing 16, Epilog USA). Microchannels were patterned onto the ~90-μm thick pressure sensitive adhesive (Adhesives Research, Ireland) layers using a knife cutter (Graphtec Corp., USA). Sacrificial valves fabricated from water dissolvable films (DFs) and cut with the knife cutter were integrated onto the single-use disc for centrifugal flow control (Gorkin et al., 2012; Nwankire et al., 2014). Five pairs of gold (working and [reference+counter]) electrodes were sputter-coated onto the bottom layer of the disc assembly (Fig. 1). The electrodes were subsequently cleaned individually by electrochemically cycling them in aqueous 0.5 M H_2SO_4 at 100 $mV\ s^{-1}$ from +0.2 V to 1.6 V vs. Ag/AgCl (3 M saturated KCl) reference

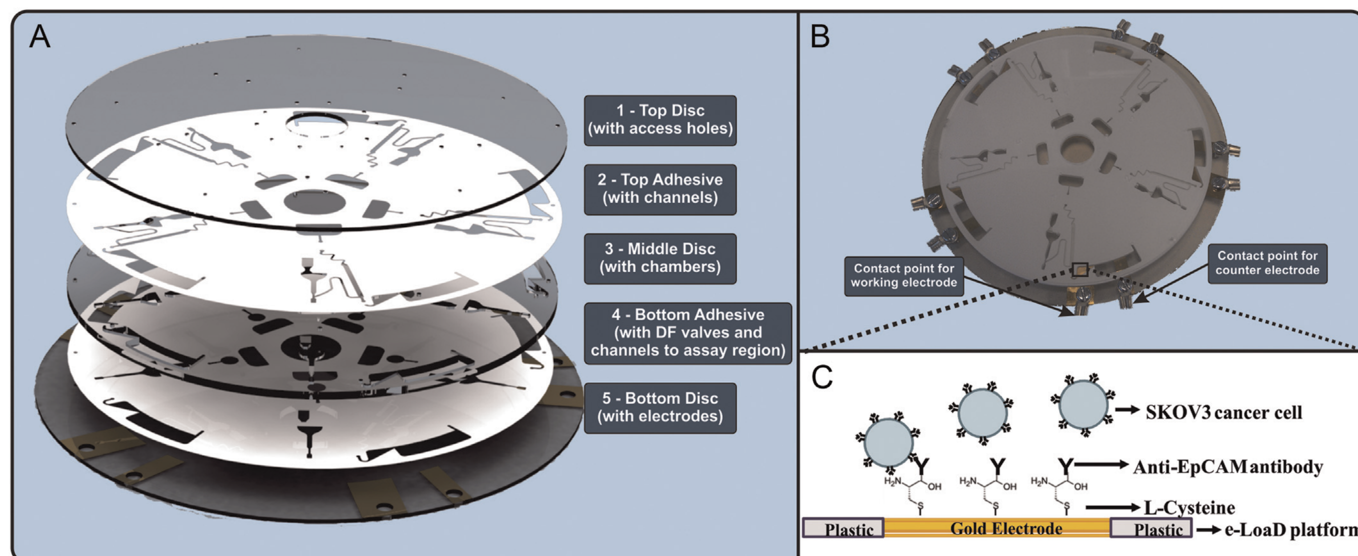

Fig. 1. (A) Rendered 3D image of the 5-layer microfluidic disc platform comprising of three 1.5-mm thick PMMA discs and two ~90-μm thick pressure sensitive adhesive films. The gold electrodes were deposited on the bottom (layer 5) disc. (B) Fully assembled disc showing contact points for the working and counter electrodes. (C) Schematic of electrochemical rare cell capture assay on polymeric eLoaD platform.

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