# A MICROFLUIDIC SYSTEM FOR THE DETECTION OF ENDOTHELIAL PROGENITOR CELLS IN BLOOD SPECIMENS USING ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY

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## ABSTRACT

This paper reports the development of a microfluidic system for rapid separation of rare endothelial progenitor cells (EPCs) in small volumes of whole blood (<100µl), as well as their selective capture and detection by immunochemistry coupled with electrochemical impedance sensing on a microelectrode array (MEA). EPCs are involved in the healing of cardiovascular injury and the EPC level in blood is linked to the assessment of cardiovascular diseases. Our system demonstrated the potential to detect EPC as low as 0.5% of peripheral blood mononuclear cells (PBMCs) directly from small-sized blood samples in a lab-on-chip setting.

**KEYWORDS:** endothelial progenitor cells (EPCs), on-chip cell purification, microelectrode array

## INTRODUCTION

Circulating EPCs are bone marrow-derived progenitor cells contributing to the repair of cardiovascular injury. Their quantity in blood serves as a biomarker for vascular function and cumulative cardiovascular diseases [1-2]. Conventional approaches to detect EPCs from blood sample, such as flow cytometry (FACS), require laborious pretreatment, long process time, and expensive equipment. Our group has previously described a highly sensitive method for EPCs detection in peripheral mononuclear cells (PBMCs) using the microelectrode array (MEA) for dielectrophresis (DEP) and electrochemical impedance spectroscopy (EIS) [3]. Due to the low concentration of circulating EPCs in blood (0.01%-1% of PBMCs), extraction and purification of EPCs from the whole blood samples has always represented a major challenge especially for a lab-on-a-chip system. Here we report a novel microfluidic methodology utilizing the principles of *in situ* blood filtration, immunomagnetic enrichment, and self-assembled antibody monolayer to selectively capture EPCs onto the MEA from small volumes of blood and followed by an EIS quantification (Fig. 1).

This system was able to rapidly separate spiked EPCs from 5 µl of diluted blood samples and concentrate EPCs onto the gold electrode surfaces. The overall separation process on the chip was completed within 30 mins, promising good potential to be a rapid point-of-care device for circulating cellular diagnosis.

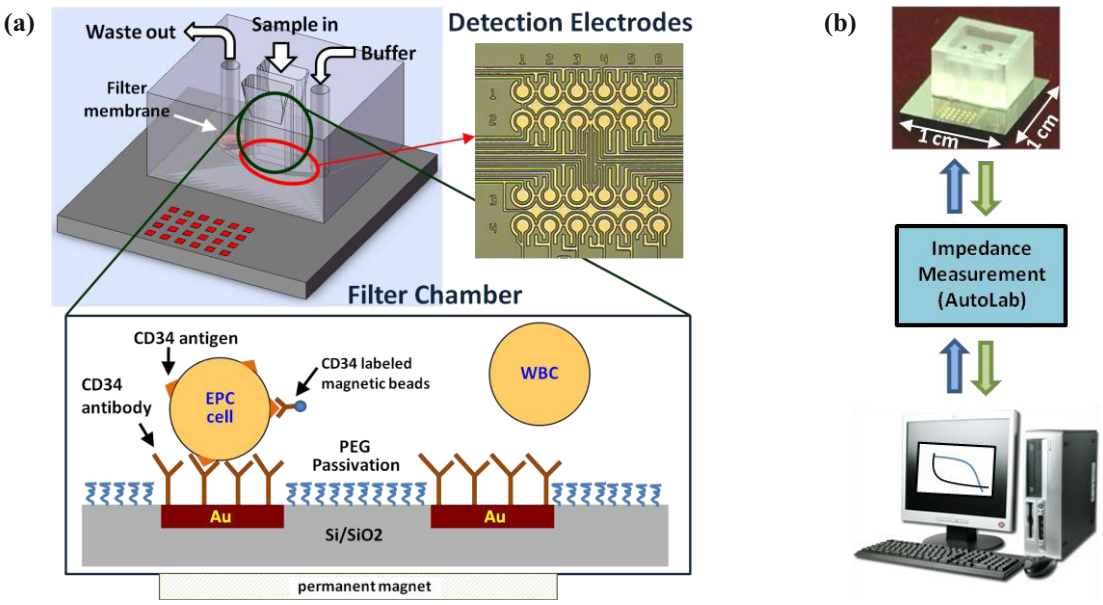


*Figure 1: (a) Setup of the microfluidic separation of EPC. An acrylic microfluidic chamber with a filter membrane (3 µm pore size) was used as a first separation step of EPCs from blood sample. EPCs extracted would be immobilized on the electrode array (top right) due to the CD34 specific antibodies (bottom). (b) Setup of impedance measurement. Changes of the electrochemical impedance were recorded at 380 kHz where the highest sensitivity to EPC attachment was observed.*

## EXPERIMENTAL

The cultured EPCs were incubated with 1 µm magnetic beads (tagged with anti-human CD34 antibodies) for 40 mins and then spiked into the diluted blood sample. An open microfluidic chamber allowed the EPC-spiked blood sample to be loaded directly from the top and two pin holes on each side were connected to two syringe pumps (Fig. 1a) for flowing the washing buffer. The sample was passed to the waste outlet through the filter membrane (3 µm pore size) that retained both PBMCs and EPCs inside the chamber.

To immobilize the CD34 antibodies onto the gold-pattern microelectrode arrays (Fig. 1a), a self-assembled functionalization process was performed: all chips were freshly cleaned by oxygen plasma before treatment ($O_2$ 60%, 80W, 100 mTorr). Submerge the chips in the mixture of 11-mercaptoundecanoic acid (MUA) and 3-mercaptopropioinic acid (MPA) to allow the formation of Au-thiol self-assembled monolayer (SAM). The rest of non-electrode area ($SiO_2$) was passivated with poly(ethylene glycol) (PEG). The 1-ethyl-3-(3-dimethylamino-propyl) cabodiimide (EDC) and N-hydroxysuccinimide (NHS) in MES buffer were used as catalyst to activate the carboxylic groups at on the end of thiol chain and finally CD34 antibodies were attached to the gold surfaces. Cy3 labeled CD34 antibody was employed to inspect the quality of the surface functionalization (Fig. 2a).

A permanent bar magnet (2.5 mm × 2.5 mm × 5 mm) was placed right underneath the MEA area in order to concentrate and attract the immunomagnetically labeled EPCs onto the sensing region. Once the EPCs were trapped on the electrodes by the antibody-antigen conjugation, changes in impedance were monitored by an impedance measuring system (Autolab) (Fig. 1). The impedance measurement on the MEA (4×6 electrode arrays, 100 µm in diameter) was scanned at 25 mV through a frequency range between 100 Hz to 1 MHz.

## RESULTS AND DISCUSSION

Fig. 2b-c shows a fluorescence analysis confirming a uniform coverage of antibody on the gold surface. The dark circular and horseshoe-shaped areas (Fig.2b) are the opened surfaces of gold electrodes before CD34 functionalization. Fig.2c confirms that those areas were filled with Cy3 labeled antibodies after the treatment. The minimal background difference between Fig. 2b and Fig. 2c indicated that the selective PEG coating on the non-electrode area repelled the antibodies from non-specific binding which in turn minimized the loss of EPCs due to non-specific binding on the SiO2 surface.

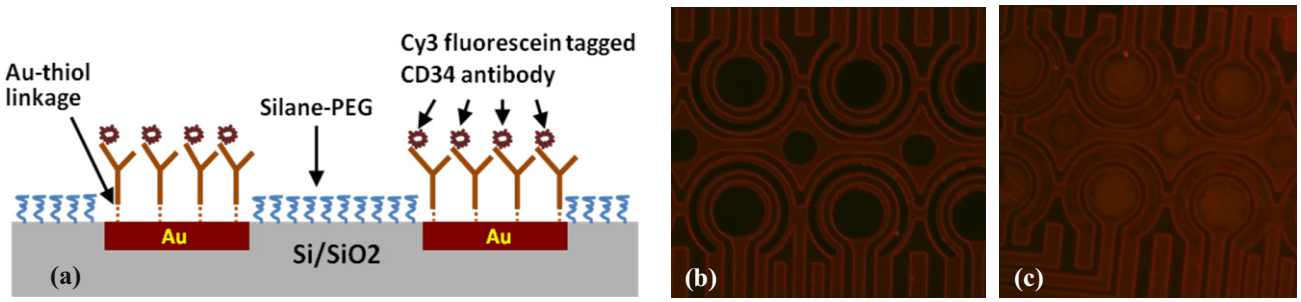


*Figure 2: The schematics of the surface chemistry on the gold and $SiO_2$ surfaces (a). Bottom images represent the fluorescent images before (b) and after (c) the surface functionalization, indicating the uniform coverage of antibody on Au electrodes.*

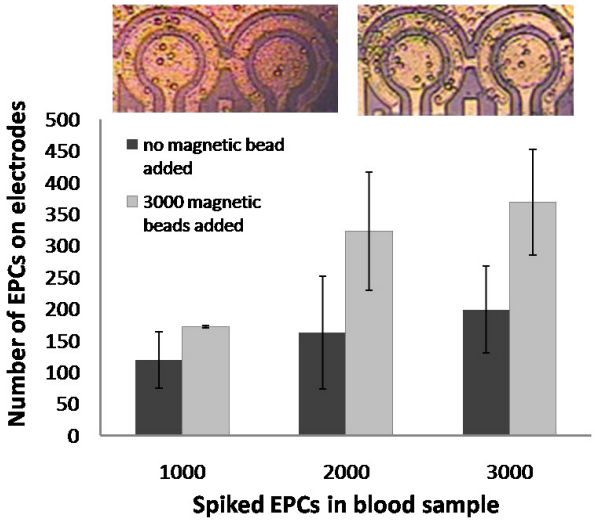


*Figure 3: EPC filtration enhanced by immunomagnetic separation. With magnetic labeling, the average trapping efficiency enhanced 80%-100% compared to the non- labeled trapping (inlet a: picture of non-labeled EPCs, b: magnetic beads labeled EPCs)*

In addition to the planar immunochemical conjugation from the immobilized antibodies, a localized magnetic field has been incorporated around the MEA region to further enhance the EPC trapping force into the bulk solution. EPCs extracted from the blood were immobilized on the gold electrodes while the rest of RBCs and PBMCs that do not express CD34 antigen were washed away. We note that the residue of unbound magnetic beads was minimal and a control test of residue beads showed negligible effect on impedance measurement at 380 kHz (data not shown). The use of immunomagnetic force enhanced the average EPC trapping efficiency by 80%-100% as compared to a non-magnetic approach (Fig. 3).

Attributing to the surface coating, negligible cell attachment outside MEA was observed (Fig.3 top inlets). EPCs on the MEA were subsequently quantified by serial impedance detection. The impedance change caused by the attachment of EPCs on the MEA of 4×6 small WEs was scanned through 100 Hz to 1 MHz. A maximum percentage increase in impedance occurred at the frequency of 380 kHz, which was used for the analysis of the electrode sensitivity (Fig. 4). Impedance increased progressively with cell number for the range between 1-25 cells. A detection limit of 6 EPCs per electrode was achieved representing about 140 cells on the MEA after blood separation.

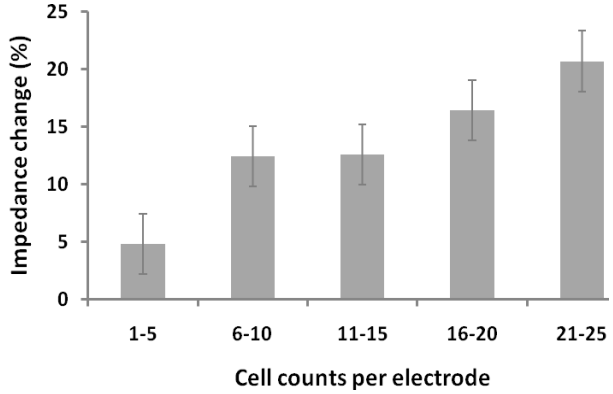


*Figure 4: Impedance signal as a function of the number of cells on the electrode. The detection limit of CD34+ cells in PBS per electrode was observed to be above 5 cells per electrode.*

## CONCLUSION

We have demonstrated a fully integrated microfluidic bioanalytical system for the purification of rare circulating EPCs from blood as well as electrochemical quantification of captured EPCs on the MEA. This sensitivity reaches the relevant range for clinical application where the EPC level can be as low as 0.5% of PBMCs (equivalent to 140 cells in 7 µl whole blood). This work may contribute to a portable bedside EPC monitoring setup where rapid results will be valuable for treatment decisions.

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