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# Developing a non-fouling hybrid microfluidic device for applications in circulating tumour cell detections

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ABSTRACT

Non-specific cell adsorption is a challenge in sensitive detections using microfluidic systems, such as detecting circulating tumour cells from blood samples. In this report, we present a new strategy to study the combined effects of surface hydrophilicity/hydrophobicity, electric charges and roughness on surface non-fouling properties of a PDMS/SU-8 microfluidic system. To achieve this, microchannel surfaces were modified by poly(amidoamine) generation 4 and generation 7, dendrimers that rendered surfaces negatively and positively charged at pH 7.4, respectively. Water contact angle, atomic force microscopy (AFM) and microscopy were used to characterize and confirm surface modifications, and the non-fouling performance of the resulting surfaces was tested using both live and dead CCRM-CEM cancer cells. Our results show that for live cells, electric charges of a surface is the most important factor affecting the non-fouling features of the surface in microfluidic systems; in contrast, for dead cells, surface hydrophilicity is the most important factor affecting surface non-fouling properties. However, surface roughness does not seem to be as important for both live and dead cells under the experimental conditions used in this study. These results also highlight the importance of different considerations when designing a lab-on-a-chip microfluidic system for high sensitivity biosensing and detection applications.



## 1. Introduction

Cancer is one of the leading causes of death around the world, greatly threatening people's health [1]. It has been reported that more than 90% of deaths in cancer patients are attributed to metastasis [2,3]. Cancer is considered a localized disease in its early stages; however, in the process of metastasis, cancer cells partly fall off from the primary tumour, entering bloodstream to become circulating tumour cells (CTCs) [4,5]. In recent years, CTCs have attracted a great deal of research interest in the field of cancer cell detection and cancer diagnosis. This is because CTCs can potentially serve as an important biomarker for the detection and diagnosis of cancers at their early stages, which if successfully implemented, can effectively improve the survival rate of many cancer patients [6,7].

In general, the detection methods of CTCs can be classified into bench-top instrument based detections and microfluidic device based detections [8]. To date, the majority of CTCs detection methods are bench-top instrument based methods such as flow cytometers [9], isolation by size of epithelial tumour cells [10], and the CellSearch system [11]. In comparison with bench-top instruments, the CTC microfluidic devices demonstrate many benefits, such as high detection sensitivity [12], low sample volume requirement, and relatively low cost for fabrication [13]. The importance of microfluidic devices with applications in chemical and biological analysis has increased dramatically in diagnostics, biochemistry and biology. For example, a poly(dimethylsiloxane) (PDMS) microfluidic device has been used to detect target cancer cells (e.g. CCRF-CEM, T-leukemia cell line) from a mixture of CCRF-CEM and background cells with a 72% detection efficiency [14]. Moreover, similar approaches have also been attempted in other studies that reported cell detection efficiencies around 80% [15,16]. Promising as they sound, the majority of CTC detection devices suffer from non-specific adsorptions, especially for CTC detections from blood samples, which is the most significant challenge for CTC detections in microfluidic devices [17,18]. This is because CTCs are extremely rare – there are only several CTCs out of $>10^9$ blood cells

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(background cells) in 1 mL of blood sample, which makes the detection and analysis of these rare cells an intractable technological challenge [19]. To solve this issue, an ultra non-fouling microfluidic system is needed for high sensitivity applications in CTC detections. For example, poly(2-acrylamido-2-methylpropane sulfonic acid) (PAMPS) brushes and poly(ethylene glycol) (PEG) have been used to render the surface non-fouling to improve the detection efficiency of specific cells from whole blood [20,21]. In addition, a smart coating, supported lipid bilayer (SLB), has also been attempted to modify device surfaces to prevent non-specific adsorption from blood samples and improve the capture efficiency of CTCs at the same time [17]. In our previous study, we developed an ultra non-fouling hybrid microfluidic device made from PDMS and SU-8 by modifying the device surfaces with poly(amidoamine) generation 7 (PAMAM-G7) dendrimer. We showed that the PAMAM-G7 surface modification of the microfluidic channels effectively enhanced their non-fouling properties by preventing non-specific adsorptions of polystyrene microbeads and formalin killed *E. coli* cells by as much as 99.7% and 95.0%, respectively. While exciting, the results from this study with model particles cannot be directly translated to applications in live CTC detections since interactions between the microfluidic surfaces and live CTCs are much more complicated than those between the microfluidic surfaces and model particles or formalin killed bacterial cells. Indeed several factors have been identified to significantly affect the non-fouling performance of a surface to prevent live cell non-specific bindings [22]. For example, it has been established that hydrophilicity of a surface is an important factor affecting non-fouling performance of the surface [23]. For instance, Thies et al. used PEG grafted silica oxide nano-particles to manipulate surface hydrophilicity in order to reduce non-specific cell (i.e. staphylococcus epidermidis HBH 276) adhesions to substrate [24]. In addition, surface electric charges are also known to be important, as live cells are negatively charged [25]. For example, it was reported that negatively charged surfaces were preferred in CTCs detection to prevent non-specific adsorptions from blood [18]. Furthermore, roughness of a surface can also affect cell adsorption to the surface [26]. Esmaeil et al. studied the effect of roughness of poly(styrene) surfaces and showed that surface roughness affected somatic stem cells adhesion to substrate surfaces [26]. However, to the best of our knowledge, no study has been done to investigate the combined effect of surface hydrophilicity/hydrophobicity, electric charges, and roughness in order to further improve surface non-fouling properties that are critically important for high sensitivity applications in circulating tumour cell detections.

In this study, PAMAM both generation 4 and generation 7 dendrimers (i.e. PAMAM-G4 and PAMAM-G7) were used to modify the surfaces within the PDMS/SU-8 hybrid microchannels. In particular, the PAMAM-G4 was fully functionalized by carboxyl groups on its surface while PAMAM-G7 was fully functionalized by amino groups on its surface, thus rendering surfaces modified by PAMAM-G4 or PAMAM-G7 negatively or positively charged in PBS (pH 7.4), respectively. This allowed us to investigate the effects of surface hydrophilicity/hydrophobicity, together with surface charges and surface roughness, on surface non-fouling properties. Our results show that for live cells, electric charges of a surface is the most important factor affecting the non-fouling properties of the surface in microfluidic systems; in comparison, for dead cells, surface hydrophilicity is the most important factor affecting surface non-fouling properties. However, for both live and dead cells surface roughness does not seem to be as important under the experimental conditions used in this study. Understanding how these surface features interact with cells or particles is very important because non-specific adsorption has been shown to significantly affect detection sensitivities [27], especially in applications where sensitivities are critically important, such as in CTC detections.

## 2. Experimental

### 2.1. Materials

PAMAM-G4 dendrimer solution (generation 4, ethylenediamine core, succinamic acid surface) (PAMAM-COOH) was obtained from Dendritech (Midland, MI). PAMAM-G7 dendrimer solution (generation 7, ethylenediamine core) (PAMAM-$NH_2$) was purchased from Sigma-Aldrich (Oakville, ON). PDMS kit (Sylgard 184) was obtained from Dow Corning (Midland, MI). SU-8 negative photoresist was purchased from MicroChem (Newton, MA). *N*-hydroxysuccinimide (NHS) and (3-Aminopropyl)-trimethoxysilane (APTMS) were obtained from Thermo Scientific Inc. (Rockford, IL). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) was purchased from Alfa Aesar (Ward Hill, MA). Toluene and Ethylenediamine anhydrous (EDA) were obtained from Fisher Scientific Inc. (Ottawa, ON). Furthermore, CCRF-CEM cells (human acute lymphoblastic leukemia, CCL-119) were purchased from American Type Culture Collection (ATCC) (Manassas, VA). All other chemicals were purchased from Sigma-Aldrich and used as received unless indicated otherwise.

### 2.2. General approach

As shown in Fig. 1, oxygen plasma is used to activate PDMS surface, and subsequently APTMS is used to aminate the plasma treated PDMS surface through silanol condensation [28]. The PDMS surface with amino functional groups is combined with SU-8 surface via reactions between amino groups and epoxy groups to prepare a microchannel. Furthermore, EDA is used to aminate the free SU-8 surface in the resulting microchannel to obtain Microchannel 1. Subsequently, PAMAM-G4 (i.e. PAMAM-COOH) is conjugated onto both PDMS and SU-8 surfaces within the microchannel simultaneously through reactions with the $-NH_2$ groups on both surfaces to obtain Microchannel 2. Finally, another layer of PAMAM-G7 (i.e. PAMAM-$NH_2$) is further immobilized onto both of the PAMAM-G4 modified PDMS and SU-8 surfaces in the microchannel to result in Microchannel 3.

### 2.3. Preparation of PDMS and SU-8 hybrid microfluidic channels

A standard soft lithography method was used to prepare the PDMS surfaces. Briefly, silicone elastomer base was well mixed with curing agent (10:1 w/w); the mixture was poured onto an SU-8 master, degassed for 30 min, and baked at 120 °C for 2 h to cure the polymer. Finally the cured PDMS was peeled off from the SU-8 master. Similarly, a bare silicon wafer (4 in.) was used to prepare PDMS surfaces without pattern. Likewise, to make an SU-8 surface without pattern, the SU-8 surface was illuminated by UV light without using a photomask.

### 2.4. Surface aminations

In order to functionalize PDMS surface with amino groups, a well-established silane coupling strategy was employed [29,30]. Briefly, oxygen plasma was used to activate PDMS surface using an Anatech SP100 plasma cleaner (Battle Creek, MI) at 100 mTorr, 118 W for 10 s. Subsequently, the oxygen plasma treated PDMS surface was reacted with APTMS solution (0.05 wt% in toluene) for 20 s to aminate the PDMS surface via silanol condensation. Finally, the APTMS treated PDMS surface was air dried at room temperature for 30 min.

In order to functionalize SU-8 surface with amino groups, the SU-8 surface was reacted with EDA solution (20 wt% in anhydrous alcohol) for 1 h and subsequently baked at 70 °C for another hour. The EDA molecule had one amino group on each side of the