

**Developing a poly(dimethylsiloxane) (PDMS)/SU-8
(negative photoresist) hybrid microfluidic system for
sensitive detection of circulating tumour cells**

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Statement of contributions of collaborators

I hereby declare that I am the sole author of this thesis. I performed all the synthesis, fabrication and cell culture experiments, and all data analysis for all the papers. Poying Yeh and Xingkai Hao helped me through discussion on this project, which inspired me a lot to design the project in chapter 3. Xiuying Yang and Jingchang Zhang gave me some suggestions and ideas in experiment design in chapter 4. The scientific guidance throughout the project and editorial comments for the written work were provided by my supervisor Dr. Xudong Cao.

Abstract

Cancer is the second leading cause of death in the world. It is therefore critically important to detect cancer in its early stage to significantly increase the survival rate of cancer patients. Circulating tumour cells (CTCs) are cancer cells that peel off from primary tumour and enter bloodstream in early stage of a cancer, and thus it has been established that these CTCs are reliable targets for early cancer diagnosis. However, background signal reduction and optimization of CTC capturing mechanisms are still significant challenges in CTC detections with high sensitivities and accuracies. To this end, we have developed an aptamers and dendrimers based ultra non-fouling microfluidic detection system for sensitive detections of circulating tumour cells.

More specifically, we demonstrate a simple strategy to bind PDMS and SU-8 surfaces in order to prepare a hybrid microfluidic device and subsequently modify both surfaces simultaneously using poly(amidoamine) (PAMAM), a highly hydrophilic dendrimer to improve non-fouling properties of the hybrid microfluidic channel. The resulting hybrid microfluidic system shows a remarkable non-specific adsorption suppression of 99.7% when tested with hydrophobic microbead suspension, an ultra non-fouling performance that has not been reported before. This is significantly important for detections with high sensitivities. X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and water contact angle are used to characterize and confirm surface modifications. In addition, we investigate the combined effects of surface properties on surface non-

fouling performance to both live and dead cells. (3-aminopropyl)-trimethoxysilane (APTMS), carboxyl functionalized PAMAM dendrimer (PAMAM-COOH) and amino functionalized PAMAM dendrimer (PAMAM-NH₂) are used to provide different surfaces with various surface hydrophilicity, electric charge and roughness. We show that electric charge of a surface is the most important factor influencing non-specific adsorption of live cells to the surface while hydrophilicity/hydrophobicity of a surface is the most important factor for dead cells. Atomic force microscopy, water contact angle and microscopy are used to characterize and confirm surface modifications. To further exploit and improve capturing efficiency of target cancer cells, we investigate the effect of the length of spacers that tether capturing aptamer to the microfluidic surfaces on capturing performance of CCRF-CEM circulating tumour cells. Aptamers with different lengths of thymine base spacers are immobilized onto PAMAM dendrimer modified surfaces in microfluidic channels. We demonstrate that ten thymine bases spacer has the best length for sgc8 aptamer to form its secondary structure for CCRF-CEM cell capture. Water contact angle, and microscopy are used to characterize and confirm surface modifications. Taken together, the results of this study significantly highlight the importance of different considerations on surface modification and its optimizations when designing a microfluidic system for high sensitivity detection and biosensing applications.

Résumé

Le cancer est la deuxième cause de mort prépondérante dans le monde. Il est donc impératif de détecter le cancer le plus tôt possible afin d'augmenter significativement le taux de survie de patients atteint de cancer. Les cellules tumorales circulantes (CTC) sont des cellules cancéreuses qui se séparent d'une tumeur pour accéder à la circulation sanguine dans un stade précoce du cancer, ce qui fait de ces CTC une cible fiable pour un diagnostic précoce du cancer. Cependant, la réduction de bruit de fond et l'optimisation mécanismes de capture de CTC pose toujours un défi important pour la détection de CTC à haute sensibilité et précision. À cette fin, nous avons développé un système de détection microfluidique ultra non-salissant à base d'aptamères et dendrimères pour la détection sensible de cellules tumorales circulantes.

Plus spécifiquement, nous avons démontré une stratégie simple pour lier des surfaces de PDMS et SU-8 afin de préparer un dispositif microfluidique hybride pour ensuite modifier simultanément les deux surfaces en utilisant la poly(amidoamine) (PAMAM), un dendrimère hautement hydrophobe afin d'augmenter les propriétés non-salissantes du canal microfluidique hybride. Le système microfluidique hybride résultant démontre une suppression d'adsorption non-spécifique remarquable de 99.7% lorsqu'il est testé avec une suspension de microbilles hydrophobes, une capacité ultra non-salissante qui n'a jamais été signalée auparavant. Ceci est de grande importance pour les détections à haute sensibilité. La spectroscopie photoélectron à rayon X (SPX), la microscopie à force atomique (MFA) et l'angle de

contacte de l'eau sont utilisés pour caractériser et confirmer les modifications de surface. De plus, nous enquêtons les effets combinatoires des propriétés de surface sur la performance non-salissante chez des cellules mortes et vivantes. Le (3-aminopropyl)-triméthoxysilane (APTMS), un dendrimère carboxylé (PAMAM-COOH) et un dendrimère fonctionnalisé aminé (PAMAM-NH₂) sont utilisés afin de fournir des surfaces avec divers niveaux d'hydrophilie, de charge électrique et de rugosité. Nous montrons que la charge électrique d'une surface est le facteur le plus important qui influence l'adsorption non-spécifique de cellules vivantes à cette surface alors que l'hydrophobicité/hydrophilie d'une surface est plus important chez les cellules mortes. La microscopie à force atomique, l'angle de contact de l'eau et la microscopie sont utilisés pour caractériser et confirmer les modifications de surface. Afin d'exploiter et améliorer davantage l'efficacité de capture de cellules cancéreuses cibles, nous enquêtons l'effet de la longueur de « spacers » qui attachent l'aptamère à la surface microfluidique sur la capacité de capturer les cellules tumorales circulantes CCRF-CEM. Des aptamères avec des aptamères à base de thymine de diverses longueurs sont immobilisés sur des surfaces modifiées avec des dendrimères PAMAM dans des canaux microfluidiques. Nous démontrons qu'un aptamère à dix bases thymine est de longueur optimale pour que l'aptamère sgc8 forme une structure secondaire essentielle à la capture de cellules CCRF-CEM. L'angle de contact de l'eau et la microscopie sont utilisés afin de caractériser et confirmer les modifications de surface. Dans leur ensemble, les résultats de cette étude mettent en évidence l'importance de différentes considérations sur la modification de surface et son optimisation en conceptualisant un système

microfluidique pour des applications en biosensing et en détection à haute sensibilité.

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List of Abbreviations

3D	Three dimensional
AFM	Atomic force microscopy
ANOVA	Analysis of variance
APTMS	(3-aminopropyl)-trimethoxysilane
ATCC	American type culture collection
ATRP	Atom transfer radical polymerization
BSA	Bovine serum albumin
CCRF-CEM	Human acute lymphoblastic leukemia cancer cells
COMSOL	Software COMSOL multiphysics
CT	Computed tomography
CTC	Circulating tumour cell
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
EDA	Ethylenediamine anhydrous
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
FAST	Fiber-optic array scanning technology
FITC	Fluorescein isothiocyanate
FRET	Fluorescence resonance energy transfer
FTIR	Fourier transform infrared spectroscopy
IDT	Integrated DNA technologies
IPN	Interpenetrating polymer network

ISET	Isolation by size of epithelial tumour cells
KJ	Kilojoule
LOD	Limit of detection
MCF	Michigan cancer foundation
MES	2-(4-morpholino)-ethane sulfonic acid
MPTMS	3-mercaptopropyltrimethoxysilane
MRI	Magnetic resonance imaging
NHS	N-hydroxysuccinimide
PAMAM	Poly(amidoamine)
PAMPS	Poly(2-acrylamido-2-methylpropane sulfonic acid)
PBS	Phosphate buffered saline
PCB	Printed circuit board
PCL	Poly(caprolactone)
PDMS	Poly(dimethylsiloxane)
PE	Poly(ethylene)
PEG	Poly(ethylene glycol)
PET	Poly(ethylene terephthalate)
PMMA	Poly(methyl methacrylate)
PP	Poly(propylene)
PPEGMA	Poly(poly(ethylene glycol)methacrylate)
PS	Poly(styrene)
PTFE	Poly(tetrafluoroethylene)

PVDF	Poly(vinylidene fluoride)
RNA	Ribonucleic acid
SELEX	Systematic evolution of ligands by exponential enrichment
SEM	Scanning electron microscopy
SLB	Supported lipid bilayer
SU-8	Negative photoresist
UV	Ultraviolet
XPS	X-ray photoelectron spectroscopy
YG	Yellow green

Chapter 1 General Introduction

1.1 Introduction

Cancer is a major public health problem and is one of the leading causes of morbidity and mortality around the world, with approximately 14 million new cases and 8 million deaths occurred in 2012 worldwide [1]. The number of new cases is estimated to increase by about 70% over the next 2 decades [2]. It has been reported that more than 90% of deaths in cancer patients are due to metastasis [3]. This is because cancer is a localized disease in its early stage; however, during the growth of primary tumour, cancer cells peel off from the primary tumour, enter bloodstream and become circulating tumour cells (CTCs) [4]. These circulating tumour cells are the hidden potential cells that will finally colonize at distant sites and form metastases (a pathogenic agent's spread from an initial or primary site to a different or secondary site within the host's body).

Traditional diagnostic methods of cancer include positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), or traditional radiography (X-ray). Among these diagnostic methods, the majority of their diagnoses are mainly base on imaging modalities of tumours, which happen in the relatively later period of cancers [5]. Compared with traditional methods, detection of circulating tumour cells will enable the diagnoses of cancers in the early period of cancers, thus significantly increasing the survival rate of cancer patients [6].

To date, the majority of CTC detection methods can be classified into microfluidic device based detections and bench-top based detections [7]. The latter are based on bench-top instruments, such as laser scanning cytometry [8-10], isolation by size of epithelial tumour cells (ISET) [11, 12], fiber-optic array scanning technology (FAST) [13, 14], high-definition fluorescence scanning microscopy [15], and flow cytometry [16-18]. Compared with bench-top based detection methods, methods based on microfluidic devices feature low sample volume requirement, superior sensitivity, reduced detection time, and relatively low cost for fabrication [15, 19]. Moreover, CTC microfluidic devices are ideal devices for point-of-care testing [20-22].

Poly(dimethylsiloxane) (PDMS) is a widely used material in the fabrication of microfluidic devices. This is because PDMS has many desirable properties such as high elasticity [23], excellent gas permeability to CO₂ and O₂ [24], moldability [25], chemical inertness [26], as well as optical transparency [27]. As another important material used in microfluidic fabrication, SU-8 (negative photoresist) has been commonly used as the material not only to prepare molds in PDMS microfluidic device fabrications but also to directly fabricate microfluidic devices. For example, it has been reported that SU-8 can be used as waveguide in optofluidic integrated applications [28, 29]. Hybrid microfluidic devices made of both SU-8 and PDMS have also attracted growing interests because they allow the preparation of complex microchannel features through an easy fabrication method [30-32]. However, bonding between the SU-8 and PDMS surfaces has proven to be much more difficult [33, 34]. Moreover, when applying the materials in microfluidic device, the

PDMS/SU-8 hybrid microfluidic system is susceptible to channel surface fouling, especially when it is used to handle samples that contain blood, proteins or cells [35]. Therefore, developing a facile approach to prepare a PDMS/SU-8 hybrid microfluidic system with an excellent surface non-fouling performance is significantly important to explore its full potentials in sensitive detection and diagnostic applications.

1.2 Hypothesis

A PDMS surface modified with PAMAM-COOH dendrimer in a microchannel will not only avoid non-specific adsorption in the microfluidic devices but also improve the detection sensitivity of the microfluidic devices.

1.3 Project objective

The overall objective of this project is to develop a non-fouling PDMS/SU-8 hybrid microfluidic system for sensitive detection of circulating tumour cells. To achieve this overall objective, three different projects have been carried out:

(1) In order to bond PDMS and SU-8 surfaces to prepare a PDMS/SU-8 hybrid microfluidic device, different strength and time of oxygen plasma was tried to active PDMS surface with hydroxyl groups. Subsequently, different concentrations of (3-

aminopropyl)-trimethoxysilane (APTMS) were investigated on bonding strength between PDMS and SU-8 surfaces. Different concentrations of ethylenediamine anhydrous (EDA) were used to study amination on SU-8 surfaces. Finally, different concentrations of poly(amidoamine) (PAMAM) were tried in PDMS/SU-8 hybrid microchannels to investigate the non-fouling performance of the microchannels modified with different concentrations of EDA solutions. Microbeads and *Escherichia coli* O157:H7 bacterial cells were used as models to evaluate the non-fouling performance of the PAMAM modified microfluidic system.

(2) The interactions between the surfaces in microfluidic device and live circulating tumour cells are much more complicated than those between the microfluidic surfaces and model particles or formalin killed bacterial cells. As a result, several properties include surface hydrophilicity/hydrophobicity, electric charge and roughness of modified PDMS surface have been investigated in order to explore the important factors that affecting non-fouling performance of the PDMS surface to live and dead circulating tumour cells. Briefly, unmodified PDMS surface provided hydrophobic surface property; Both of PAMAM-NH₂ (Generation 4) and PAMAM-COOH (Generation 7) provided hydrophilic surface property; APTMS and PAMAM-NH₂ (Generation 4) provided positive electric charge while PAMAM-COOH (Generation 7) provided negative electric charge; and different surfaces provided various roughness at the same time. CCRF-CEM (human acute lymphoblastic leukemia) cancer cells were used as models to evaluate the non-fouling performance of the modified surfaces.

(3) In CTC detection, when introducing capture ligands (e.g. aptamers, antibodies) onto solid surfaces, spacers are generally needed to help the immobilized capture ligands overcome steric interference and finally improve the capturing performance of the ligands. In order to investigate the effects of spacer length of capturing ligands on CTC capture performance, different lengths of spacers were used in this study. Briefly, same capture ligands were linked with 2, 5, 10 or 20 thymine bases as spacers. Subsequently, the molecules were immobilized onto PAMAM modified PDMS surfaces through the reactions between the amino groups on the end of spacers and carboxyl groups on PAMAM modified PDMS surfaces. Finally, performances of the capturing ligands with different lengths of spacers were evaluated using circulating tumour cells in microfluidic channels. CCRF-CEM (human acute lymphoblastic leukemia) cancer cells were used as models of circulating tumour cells in this study.

1.4 The organization of the thesis and the importance of scientific contributions

Based on the project objectives, the thesis is consisted of six chapters. Chapter 1 and Chapter 6 are about general introduction, general discussion, conclusions, recommendations and contributions, respectively related to the entire thesis. Chapter 2 is an overview of the development of non-fouling modification methods of PDMS surface and related techniques studied in this project. Chapter 3, 4 and 5 are

independent research publications published or submitted to the referred scientific journal.

Chapter 3 Yubo Qin, Poying Yeh, Xingkai Hao, Xudong Cao. Developing an ultra non-fouling SU-8 and PDMS hybrid microfluidic device by poly(amidoamine) engraftment. Journal of Colloids and Surfaces B: Biointerfaces, 127 (2015) 247-255.

In this study, a new approach to prepare an ultra non-fouling poly(dimethylsiloxane) (PDMS)/SU-8 hybrid microfluidic system was developed with simultaneously modifying both PDMS and SU-8 surfaces with poly(amidoamine) (PAMAM) dendrimers. PAMAM dendrimers were introduced to provide highly hydrophilic surfaces in order to enhance non-fouling properties of the hybrid microfluidic channel. To characterize the changes of surface during the surface modifications, different surface characterization techniques, such as water contact angle, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) were employed in this study. Microbeads and *Escherichia coli* O157:H7 bacterial cells were used as models to evaluate the non-fouling performance of the PAMAM modified microfluidic system. Results demonstrated that the obtained hybrid microfluidic system showed a significant adsorption suppression of 99.7% and 95% for microbead and bacteria, respectively.

Chapter 4 Yubo Qin, Xiuying Yang, Jingchang Zhang, Xudong Cao. Developing a non-fouling hybrid microfluidic device for applications in circulating tumour cell detections. Journal of Colloids and Surfaces B: Biointerfaces, 151 (2017) 39-46.

In this study, a new strategy was developed to investigate 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 PAMAM generation 4 and generation 7, dendrimers that rendered surfaces positively and negatively charged at pH 7.4, respectively. Water contact angle, atomic force microscopy (AFM) and microscopy were employed to characterize and confirm surface modifications, and the non-fouling performance of the resulting surfaces was tested using both live and dead CCRF-CEM (human acute lymphoblastic leukemia) cancer cells. Results showed that for live cells, electric charges of a surface were the most important factor affecting the non-fouling features of the surface in microfluidic systems; in contrast, for dead cells, surface hydrophilicity was the most important factor affecting surface non-fouling properties. However, surface roughness did not seem to be as important for both live and dead cells under the experimental conditions used in this study.

Chapter 5 Yubo Qin, Xudong Cao. Effects of aptamer spacer length on circulating tumour cell detection in microfluidic systems.

In this study, we investigated the effect of spacers via which capturing aptamers were immobilized on a PDMS/SU-8 microfluidic system. To achieve this, capturing aptamers were tethered to the microchannel surfaces through different lengths of thymine bases as spacers that allowed aptamers to form their secondary structures. Water contact angle, RNAstructure simulation and microscopy were employed to characterize and confirm surface modifications, and aptamer secondary structures. Finally, cell capturing performances of the resulting surfaces were tested using live CCRF-CEM cancer cells. Our results showed that ten thymine bases spacer had the best length as a spacer for sgc8 aptamer for CCRF-CEM cell capture under the experimental conditions used in this study. Spacers shorter than ten thymine bases will result in lack of space for the aptamers that limiting their ability to capture target cells. When the spacers of the aptamers are in an excessive length, the secondary structure formation of partial aptamers will change and lose their capture ability to capture target cells.

1.5 References

- [1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2016, CA: a cancer journal for clinicians, 66 (2016) 7-30.
- [2] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet - Tieulent, A. Jemal, Global cancer statistics, 2012, CA: a cancer journal for clinicians, 65 (2015) 87-108.
- [3] C. Alix-Panabières, K. Pantel, Challenges in circulating tumour cell research, Nature Reviews Cancer, 14 (2014) 623-631.
- [4] M. Liu, M. Tang, M. Li, F. Gao, C. Shi, J. Hou, W. Zeng, Circulating Tumor Cells: A New Window for Diagnosis and Evaluation of Cancer, Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 16 (2016) 1529-1540.
- [5] M.-Y. Huang, H.-L. Tsai, J.-J. Huang, J.-Y. Wang, Clinical implications and future perspectives of circulating tumor cells and biomarkers in clinical outcomes of colorectal cancer, Translational oncology, 9 (2016) 340-347.
- [6] M. Ilie, V. Hofman, E. Long-Mira, E. Selva, J.-M. Vignaud, B. Padovani, J. Mouroux, C.-H. Marquette, P. Hofman, "Sentinel" circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease, PloS one, 9 (2014) e111597.
- [7] B. Hong, Y. Zu, Detecting circulating tumor cells: current challenges and new trends, Theranostics, 3 (2013) 377.
- [8] C. Bayarri-Lara, F.G. Ortega, A.C.L. de Guevara, J.L. Puche, J.R. Zafra, D. de Miguel-Pérez, A.S.-P. Ramos, C.F. Giraldo-Ospina, J.A.N. Gómez, M. Delgado-Rodriguez, Circulating tumor cells identify early recurrence in patients with non-small cell lung cancer undergoing radical resection, PloS one, 11 (2016) e0148659.
- [9] K. Tjensvoll, O. Nordgård, R. Smaaland, Circulating tumor cells in pancreatic cancer patients: methods of detection and clinical implications, International journal of cancer, 134 (2014) 1-8.
- [10] K. Pachmann, O. Camara, A. Kavallaris, S. Krauspe, N. Malarski, M. Gajda, T. Kroll, C. Jorke, U. Hammer, A. Altendorf-Hofmann, Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse, Journal of clinical oncology, 26 (2008) 1208-1215.
- [11] P. Pinzani, B. Salvadori, L. Simi, S. Bianchi, V. Distanti, L. Cataliotti, M. Pazzagli, C. Orlando, Isolation by size of epithelial tumor cells in peripheral blood of patients with breast cancer: correlation with real-time reverse transcriptase-polymerase chain reaction results and feasibility of molecular analysis by laser microdissection, Human pathology, 37 (2006) 711-718.
- [12] N.S. Wong, H.J. Kahn, L. Zhang, S. Oldfield, L.-Y. Yang, A. Marks, M.E. Trudeau, Prognostic significance of circulating tumour cells enumerated after filtration enrichment in early and metastatic breast cancer patients, Breast cancer research and treatment, 99 (2006) 63-69.
- [13] R.T. Krivacic, A. Ladanyi, D.N. Curry, H. Hsieh, P. Kuhn, D.E. Bergsruud, J.F. Kepros, T. Barbera, M.Y. Ho, L.B. Chen, A rare-cell detector for cancer, Proceedings of the

National Academy of Sciences of the United States of America, 101 (2004) 10501-10504.

[14] H.B. Hsieh, D. Marrinucci, K. Bethel, D.N. Curry, M. Humphrey, R.T. Krivacic, J. Kroener, L. Kroener, A. Ladanyi, N. Lazarus, High speed detection of circulating tumor cells, *Biosensors and Bioelectronics*, 21 (2006) 1893-1899.

[15] D. Marrinucci, K. Bethel, A. Kolatkar, M.S. Luttgen, M. Malchiodi, F. Baehring, K. Voigt, D. Lazar, J. Nieva, L. Bazhenova, Fluid biopsy in patients with metastatic prostate, pancreatic and breast cancers, *Physical biology*, 9 (2012) 016003.

[16] E. Racila, D. Euhus, A.J. Weiss, C. Rao, J. McConnell, L.W. Terstappen, J.W. Uhr, Detection and characterization of carcinoma cells in the blood, *Proceedings of the National Academy of Sciences*, 95 (1998) 4589-4594.

[17] M. Takao, K. Takeda, Enumeration, characterization, and collection of intact circulating tumor cells by cross contamination - free flow cytometry, *Cytometry Part A*, 79 (2011) 107-117.

[18] B. Hong, M. Mace, R. Crowder, T. Coleman, W. He, F. Szczepanski, J. Feczko, J. Lyzak, Metastatic breast cancer detection and therapy monitoring using folate-targeting flow cytometry, *Journal of Clinical Oncology*, 29 (2011) 23-23.

[19] J. Kepros, T. Barbera, M.Y. Ho, L.B. Chen, R.A. Lerner, R.H. Bruce, A rare-cell detector for cancer, *Proc Natl Acad Sci USA*, 101 (2004) 10501-10504.

[20] S.L. Stott, C.-H. Hsu, D.I. Tsukrov, M. Yu, D.T. Miyamoto, B.A. Waltman, S.M. Rothenberg, A.M. Shah, M.E. Smas, G.K. Korir, Isolation of circulating tumor cells using a microvortex-generating herringbone-chip, *Proceedings of the National Academy of Sciences*, 107 (2010) 18392-18397.

[21] U. Dharmasiri, S. Balamurugan, A.A. Adams, P.I. Okagbare, A. Obubuafo, S.A. Soper, Highly efficient capture and enumeration of low abundance prostate cancer cells using prostate - specific membrane antigen aptamers immobilized to a polymeric microfluidic device, *Electrophoresis*, 30 (2009) 3289-3300.

[22] H.K. Lin, S. Zheng, A.J. Williams, M. Balic, S. Groshen, H.I. Scher, M. Fleisher, W. Stadler, R.H. Datar, Y.-C. Tai, Portable filter-based microdevice for detection and characterization of circulating tumor cells, *Clinical Cancer Research*, 16 (2010) 5011-5018.

[23] K.-P. Zimmer, A. Braun, M. Kostrzewa, Shrinking the apparatus size for DNA analysis, *Smart Electronics and MEMS II*, International Society for Optics and Photonics, 2001, pp. 58-66.

[24] C. Séguin, J.M. McLachlan, P.R. Norton, F. Lagugné-Labarthe, Surface modification of poly (dimethylsiloxane) for microfluidic assay applications, *Applied Surface Science*, 256 (2010) 2524-2531.

[25] S. Oh, Thick single-layer positive photoresist mold and poly (dimethylsiloxane)(PDMS) dry etching for the fabrication of a glass-PDMS-glass microfluidic device, *Journal of Micromechanics and Microengineering*, 18 (2008) 115025.

[26] P.N. Nge, C.I. Rogers, A.T. Woolley, Advances in microfluidic materials, functions, integration, and applications, *Chemical reviews*, 113 (2013) 2550-2583.

- [27] Q. Tu, J.-C. Wang, Y. Zhang, R. Liu, W. Liu, L. Ren, S. Shen, J. Xu, L. Zhao, J. Wang, Surface modification of poly (dimethylsiloxane) and its applications in microfluidics-based biological analysis, (2012).
- [28] P.-Y. Yeh, N.A. Rossi, J.N. Kizhakkedathu, M. Chiao, A silicone-based microfluidic chip grafted with carboxyl functionalized hyperbranched polyglycerols for selective protein capture, *Microfluidics and Nanofluidics*, 9 (2010) 199-209.
- [29] E. Zeller, A. Mitchell, Sealed air-core planar waveguide arrays in SU8 epoxy, *Micro-and Nanotechnology: Materials, Processes, Packaging, and Systems IV*, International Society for Optics and Photonics, 2009, pp. 72690E.
- [30] C.-F. Wan, C.-S. Chen, K.-C. Hwang, Y.-H. Lai, J.-Y. Luo, P.-C. Liu, L.-S. Fan, Development and automation of microelectromechanical systems-based biochip platform for protein assay, *Sensors and Actuators B: Chemical*, 193 (2014) 53-61.
- [31] M.S. Cheri, H. Latifi, J. Sadeghi, M.S. Moghaddam, H. Shahraki, H. Hajghassem, Real-time measurement of flow rate in microfluidic devices using a cantilever-based optofluidic sensor, *Analyst*, 139 (2014) 431-438.
- [32] F. Perdignes, J.M. García, J.M. Quero, Fabrication process for integration of SU-8 check valves on printed circuit board-based microfluidic platforms, *Micro & Nano Letters*, 9 (2014) 37-40.
- [33] Z. Zhang, P. Zhao, G. Xiao, B.R. Watts, C. Xu, Sealing SU-8 microfluidic channels using PDMS, *Biomicrofluidics*, 5 (2011) 046503.
- [34] C.-W. Tsao, D.L. DeVoe, Bonding of thermoplastic polymer microfluidics, *Microfluidics and Nanofluidics*, 6 (2009) 1-16.
- [35] Q. Tu, J.-C. Wang, R. Liu, J. He, Y. Zhang, S. Shen, J. Xu, J. Liu, M.-S. Yuan, J. Wang, Antifouling properties of poly (dimethylsiloxane) surfaces modified with quaternized poly (dimethylaminoethyl methacrylate), *Colloids and Surfaces B: Biointerfaces*, 102 (2013) 361-370.

Chapter 2 Literature Review

2.1 Introduction

In this chapter, the background knowledge related to this project is reviewed. First, the modifications of PDMS surface to prepare non-fouling surfaces are reviewed. Subsequently, the capture ligands and spacers for rare cell detection are reviewed.

2.2 Modification of PDMS to prepare non-fouling surfaces

2.2.1 Microfluidic systems and poly(dimethylsiloxane) (PDMS)

The non-fouling property of the materials used in detection applications is always an important main factor that influencing the efficiency of the detection performance. This is because in these detection applications, the number of numerous background particles or cells is exponentially larger than that of target particles or cells. For example, in blood sample of applications for cancer cell detection, the concentrations of background cells such as red blood cells, white blood cells and platelets are 10^9 , 10^7 and 10^8 cells/mL, respectively. However, the concentration of target cancer cells is about 1 to 10 cells/mL [1].

Among the detection systems, microfluidic systems with applications in chemical and biological analysis have significantly attracted growing research interests in diagnostics, biology and biochemistry [2]. This is because microfluidic systems

demonstrate many benefits such as high sensitivity [3], rapid reaction, reduced sample volume requirement [4], low cost to fabricate and low power consumption [5]. With the advantages provided by microfluidic systems, high-sensitivity detections based on microfluidic chips have been widely used and have shown a bright outlook for applications with high sensitivities and high accuracies [6-11].

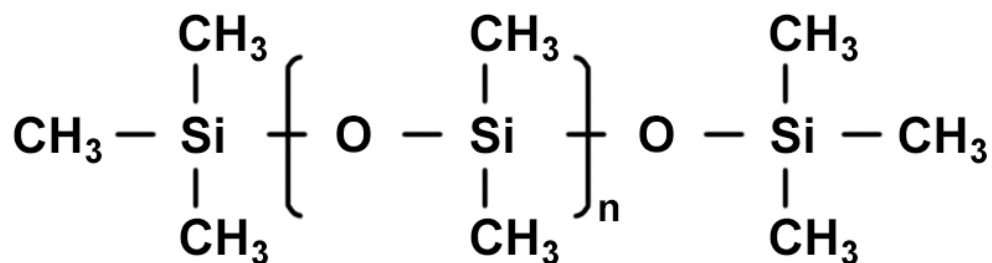


Figure 2-1. Chemical structure of poly(dimethylsiloxane) (PDMS), where n is the number of repeating monomer $[\text{SiO}(\text{CH}_3)_2]$ units.

Poly(dimethylsiloxane) (PDMS) is a widely used material in microfluidic device fabrications because it offers many beneficial properties, such as moldability [12], chemical inertness [13], optical transparency [14], excellent gas permeability to CO_2 and O_2 , as well as high elasticity [15]. As a result, PDMS have been used as an important material in many areas such as food inspection, biomedical engineering, biochemical engineering and chemical engineering [16-21]. The chemical structure

of PDMS is shown as Figure 2-1, where n represent the number of $\text{SiO}(\text{CH}_3)_2$ repeating monomer units.

2.2.2 Soft lithography and microfluidic device fabrication

Soft lithography is the most commonly used method to create PDMS structures. A master, or a mold is required in the process of microfluidic device fabrication and generally this master is created by photolithography. The master provides the hollow space of the microchannels, where the samples will eventually flow.

In order to produce the master for PDMS microfluidic device fabrication, photoresist is used in photolithography. SU-8 (negative photoresist) is a widely used epoxy-based photoresist in the production of microfluidic master. Negative photoresist indicates a photoresist that the parts treated by UV generate crosslinking, while the remaining parts of the photoresist remains soluble and can be washed off from the substrate in the step of development. The chemical structure of SU-8 photoresist is shown as Figure 2-2.

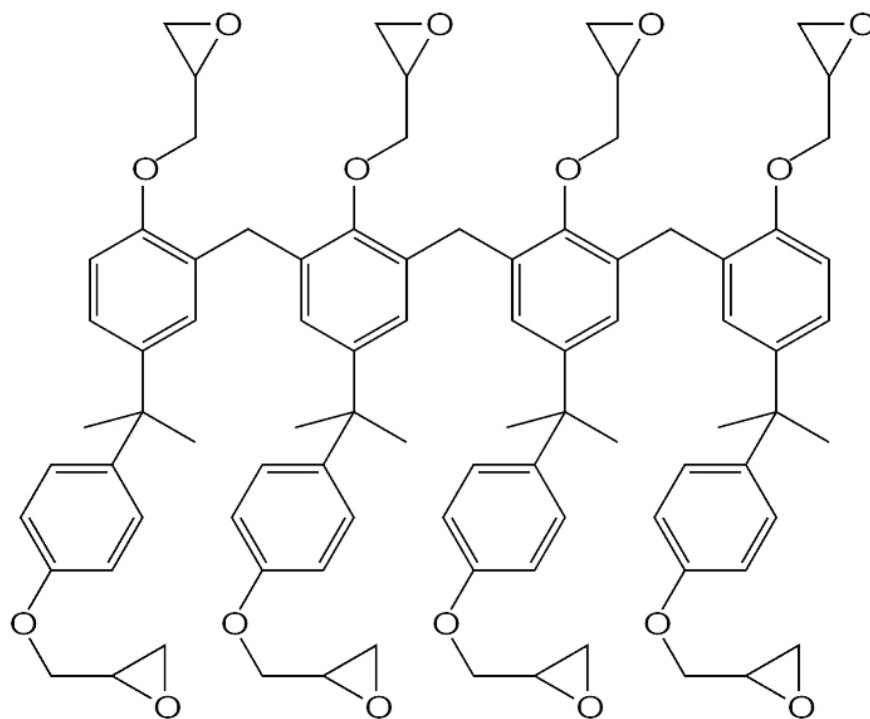


Figure 2-2. Chemical structure of SU-8 (negative photoresist).

In order to produce the SU-8 master for microfluidic device fabrication, SU-8 negative photoresist is poured over a silicon wafer and spun at certain speed to make the surface of photoresist flat and even. The spin speed will also determine the thickness of the SU-8 photoresist whereby determine the final depth of the microchannels. As it is shown in Figure 2-3, after spinning the SU-8 photoresist, a piece of high-resolution photomask is placed on top of the SU-8 photoresist. Subsequently, the material is treated by UV and followed by baking steps to cure the photoresist into solid phase. This is followed by a washing step with SU-8 developer to remove the uncured SU-8 photoresist while the cured photoresist remains on the silicon wafer and becomes the master of PDMS microchannel fabrication.

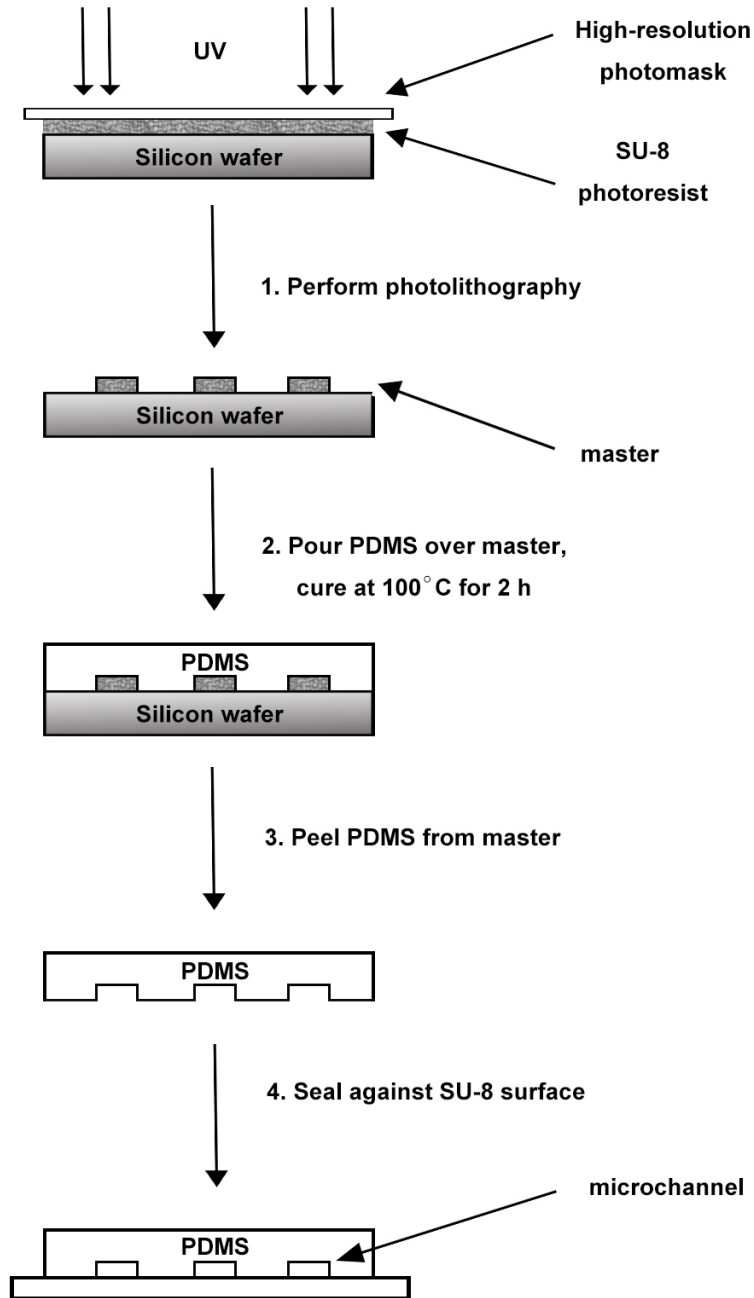


Figure 2-3. Fabrication process of PDMS and SU-8 hybrid microfluidic device.

PDMS is formed by mixing PDMS elastomer base with its curing agent for several minutes. The mixture is subsequently poured onto the SU-8 master and cured to harden, after which PDMS can be peeled away from the master without damage to both of the PDMS and SU-8 master. This makes the master mold reusable in the future. The resulting PDMS with hollow microchannels can be sealed with various substrates by using different chemical binding methods. As shown in Figure 2-3, this method makes it possible to produce microfluidic devices used in the areas of sensitive detection, food inspection and biomedical research. The resolution of the resulting microchannels and the designed patterns of the microfluidic devices depend on the resolution of the photomask and the highest resolution of this method can reach as fine as 6 nm [22].

Initially, the majority of SU-8 applications were mainly on the preparation of microfluidic devices as a material of masters [23]. Moreover, SU-8 photoresist offers many advantages such as highly cross-linked matrix and aromatic functionality, which makes SU-8 photoresist extremely chemically inert and thermally stable [24, 25]. It can withstand sodium hydroxide, acetone and nitric acid at high temperature, and it is more resist to prolonged plasma etching [26, 27]. In addition, SU-8 can provide unique optical properties in microfluidic systems [28]. As a result, it has attracted increasing research interests to use SU-8 directly as the main material in the fabrication of microfluidic devices in recent years [28-30]. For example, Kotari et al. used SU-8 as material in a microfluidic device for particle sorting [28]. With

higher refractive index, SU-8 served as a waveguide concentrating laser beam with less scattering in the optofluidic integrated device. Watts et al. used SU-8 as material in an SU-8-based photonic-microfluidic integrated device for scattering detection and counting application [30].

Hybrid microfluidic systems made of both PDMS and SU-8 have also shown their increasing significance in the areas of chemical and biomedical engineering because both of PDMS and SU-8 can be fabricated in easy process to prepare complex microchannel features [4, 31, 32]. Perdigones et al. developed a fabrication strategy to integrate SU-8 check valves within a printed circuit board (PCB) microfluidic system [32]. Integration of SU-8 check valves and fabrication of the device with PCB could be done simultaneously and obtain the PCB/SU-8 hybrid microfluidic device without need of assembly. However, it has been proven that the bonding between PDMS and SU-8 surfaces is much more difficult [33, 34]. In addition, the hydrophilicity characterizations of PDMS and SU-8 surfaces show that the water contact angles of PDMS and SU-8 surfaces are relatively high, indicating that the surfaces of the materials are easy to be fouled. After polymerization, PDMS surface structure keeps PDMS surface hydrophobic from being wetted by polar solvent such as water, and cause hydrophobic contaminants adsorption. As a result, many studies are focused on modifying PDMS surfaces into hydrophilic to make the surfaces non-fouling for various applications.

2.2.3 Plasma treatment to PDMS surface

In order to modify PDMS surface property into hydrophilic, plasma treatment is a widely used method. It can be used to either change the functional groups on PDMS surface to directly achieve property modification or change the functional groups on PDMS surface for further modification by hydrophilic molecules. For example, oxygen plasma can be performed on PDMS surface to introduce hydroxyl functional groups onto the surface. With the treatment by oxygen plasma, the water contact angle of treated PDMS surface decreases to around 40 ° from its original 110 ° [2]. The treatment to PDMS surface by oxygen plasma makes the PDMS surface hydrophilic to avoid non-specific adsorptions of hydrophobic particles. Meanwhile, the hydroxyl functionalized PDMS surface can be used for further surface modifications by different hydrophilic molecules in various applications. However, it should be noted that the hydroxyl functionalized PDMS surfaces keep stable for less than 30 minutes, after which the surface will subsequently undergo a hydrophobic recovery phenomena [35]. The treatment of oxygen plasma to PDMS surface should be performed at an appropriate exposing power for an appropriate period. High power and long period of oxygen plasma exposure will result in damage to backbone structure of PDMS and efficacy loss of the treatment.

In addition, nitrogen plasma is commonly used to introduce amino functional groups onto PDMS surfaces [36]. This method can be used in the applications in

which the functional groups on the molecules for modification in next step are more suitable to react with amino functional groups rather than hydroxyl functional groups. Except for oxygen plasma and nitrogen plasma, carbon dioxide plasma and have also been used to modify polymer surface properties. As it is shown in Table 2-1, oxygen plasma is generally used to introduce functional groups containing oxygen to polymer surfaces such as poly(dimethylsiloxane) (PDMS) [37], polyethylene terephthalate (PET) [38], polyethylene (PE) [39] and polycaprolactone (PCL) [40]. Nitrogen plasma is mainly used to impart amino functional groups to the surface of PDMS [36] and polystyrene (PS) [41], and ammonia plasma can be used to introduce amino functional groups to the surface of poly(tetrafluoroethylene) (PTFE) [42-44]. In addition to oxygen, nitrogen and ammonia plasma, carbon dioxide plasma is generally applied to impart carboxyl groups on PE [45-48], PS [41] and polypropylene (PP) surfaces [45]. Moreover, poly(methyl methacrylate) (PMMA) can be oxidized by air plasma [49, 50] and inert gases are generally used to impart radical sites on polymer surfaces for the following graft copolymerization. For example, Cheng et al., Ademovic et al. and Kang et al. have used argon (Ar) plasma to treat PCL, polyvinylidene fluoride (PVDF) and PTFE surfaces respectively in order to graft polymerized acrylic acid [51-53]. Ar plasma treatment has also been used in 4-vinylpyridine and polymerize poly(ethylene glycol) engraftment on PVDF, PET and PE surfaces [54, 55].

Table 2-1. Summary of plasma treatment and corresponding imparted functional groups.

Plasma Gas	Treated Surfaces	Functionalization
Oxygen	Poly(dimethylsiloxane) (PDMS)	Functional groups containing oxygen
	Polyethylene terephthalate (PET)	
	Polyethylene (PE)	
	Polycaprolactone (PCL)	
Nitrogen	Poly(dimethylsiloxane) (PDMS)	Amino groups
	Polystyrene (PS)	
Ammonia	Poly(tetrafluoroethylene) (PTFE)	
Carbon dioxide	Polyethylene (PE)	Carboxyl groups
	Polystyrene (PS)	
	Polypropylene (PP)	
Air	Poly(methyl methacrylate) (PMMA)	Carbonyl groups
Inert gas	Polycaprolactone (PCL)	Introduce radical sites
	Polyvinylidene fluoride (PVDF)	
	Poly(tetrafluoroethylene) (PTFE)	
	Polyethylene terephthalate (PET)	
	Polyethylene (PE)	

2.2.4 Amination of PDMS surface

In many applications, PDMS surface amination is needed after the treatment of plasma. On one hand, surface amination can introduce amino functional groups on PDMS surface to provide functionality in order to react with other surface modification materials in the future. On the other hand, comparing with introducing amino functional groups by nitrogen and ammonia plasma, amino groups introduced by silanol condensation are more stable and have much longer time before hydrophobic recovery phenomena [56].

3-mercaptopropyltrimethoxysilane (MPTMS) and 3-aminopropyltrimethoxysilane (APTMS) are commonly used material to impart amino functional groups with high stabilities [36, 57-61]. After the treatment of oxygen plasma, the hydroxyl groups on activated PDMS surface can react with APTMS to generate a second layer with high stability through silanol condensation. The APTMS modified PDMS surface have been proved to be stable without hydrophobic recovery for more than two weeks and water contact angle of the APTMS modified PDMS surface is about 50° compared with its original 110° [56]. After the modification by APTMS, the functional groups on PDMS surface are amino groups, which can react with molecules ending with carboxyl groups to achieve further surface modifications in surface non-fouling applications.

2.2.5 Approaches to increase PDMS surface hydrophilicities

In this part, approaches to increase hydrophilicities of PDMS surfaces in various applications are reviewed. Vickers et al. believe that diffusion of unreacted oligomer to PDMS surface is the reason for the quick hydrophobic recovery after plasma treatment [62]. The group developed a simple method to fabricate a hydrophilic PDMS surface with long-term stability in air to prevent non-specific particles adsorptions. First, a series of solvents have been used to treat PDMS surface to remove excess oligomers from the surface. Second, the resulting PDMS surface was treated by air plasma to generate a layer of hydrophilic SiO₂ with high stability. Compared with traditional plasma methods, this method provides hydrophilic property with long-term stability to PDMS surface, which is significantly important for the studies using hydrophilic PDMS surfaces for non-fouling applications. Liu et al. studied and proved that ionic polymers could be used in microfluidic applications to avoid non-specific adsorptions in microfluidic channels [63]. Kyung et al. reported a PDMS microfluidic device modified by polyelectrolyte multilayers to prevent non-specific analytes adsorption [64]. The structure of the microfluidic device contains a frits integrated bead chamber to retain particles and an injector to introduce sample. The study found that BODIPY, a neutral lipid compound, was strongly absorbed on unmodified PDMS surface, while the polyelectrolyte multilayers modified surface could effectively prevent the adsorption of the lipid compound.

In addition, Wirth et al. and Gezer et al. presented a method to modify PDMS surfaces with polyacrylamide brushes and the result showed that non-specific adsorption of lysozyme was significantly prevented [65-67]. In this study, the modified PDMS surface showed a 10-fold improvement on preventing irreversible lysozyme adsorption, compared with unmodified glass surface, and a 20-fold enhancement compared with unmodified PDMS surface. Moreover, water contact angles of the surfaces have been characterized and the modified PDMS surface can be stable without hydrophobic recovery phenomena for at least 1 month. Tugulu et al. reported a new method to modify PDMS surface with poly (poly(ethylene glycol)methacrylate) (PPEGMA) brushes in order to reduce non-specific protein adsorptions [68]. In this study, Tugulu used a method of vapour hydrolysis/deposition cycles to generate an interpenetrating polymer network (IPN) functionalized with surface-confined atom transfer radical polymerization (ATRP)-initiator. Different with the majority of the methods that have been reported to conjugate this polymer layers on PDMS surfaces, this method obviates the need of pretreatment to PDMS surface. The result showed that the IPN layer functionalized by surface-confined ATRP-initiator were successfully used to generate PPEGMA brushes in the strategy and that the PPEGMA modified PDMS surface significantly prevented non-specific adsorption of protein as compared to unmodified PDMS surface. While interesting, these methods are complicated and the effects are inefficient. For example, the polyelectrolyte multilayers modified device and the

ionic polymers treated device only showed short-term non-specific prevention ability because of a quick recover rate of water contact angle.

In addition, three research groups developed PDMS microfluidic devices with higher level of non-fouling performance by using various surface modification methods. Because non-specific adsorption of dye molecules will increase background level of fluorescence and limit concentration of fluorescent molecules in microfluidic systems, Sasaski et al. reported a modification method by poly-*p*-xylylene derivatives (parylenes) conformal deposition in order to prevent non-specific adsorption of Rhodamine B [69]. Other research have found that the parylene modified PDMS surfaces could also effectively avoid non-specific adsorptions of DNA and protein molecules [70, 71]. Zhang et al. have used 3-chloropropyltrichlorosilane and 3-glycidoxypropyltrimethoxysilane to treat oxygen plasma activated PDMS surfaces, this is followed by further modifications to the surfaces with alkyne-PEG and NH₂-PEG linear polymers [72, 73]. Fourier transform infrared spectroscopy (FTIR) was used to characterize the resulting surfaces and demonstrating two adsorption characteristic peaks located at around 2878 cm⁻¹ and 1336 cm⁻¹, which are consistent with the -CH₂O- groups of PEG. Water contact angles have also been measured and result showed that compared with 110 ° of unmodified PMDS surface, the water contact angles of alkyne-PEG modified PDMS surface and NH₂-PEG modified PDMS surface were around 65 ° and 71 °, respectively. Storage experiments have also been performed and results

demonstrated that the hydrophilicities of these linear polymer modified surfaces could maintain for more than 1 month.

Recently, Yeh et al. found a simple non-fouling strategy by using PEG linear polymer to modify PDMS surface in microfluidic channels in order to prevent non-specific adsorption of model particles and bovine serum albumin (BSA) [36]. In this study, PDMS surface was first pretreated by air plasma and then APTMS was used to aminate the plasma-activated PDMS surface. Subsequently, mPEG-NHS (*N*-hydroxysuccinimide) was used to modify the aminated PDMS surface through the reactions between amino functional groups on PDMS surface and NHS groups of mPEG-NHS molecules. Surface properties and modifications were characterized and confirmed by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and water contact angle. Finally, non-fouling performance of this surface modification strategy was tested by polystyrene microbeads and BSA in microfluidic devices. Results of the tests showed that the PEG-modified PDMS surface could prevent 65% and 92% non-specific adsorption of BSA and polystyrene microbeads, respectively. Moreover, in this study, the author pointed out that longer PEG chains and highly branched dendrimers could be used as materials to further improve the non-fouling performance of microfluidic devices. This is because longer PEG chains provide relatively bigger hydration shells and highly branched dendrimers have higher chain flexibility and extension [61, 74]. Steric interactions of chains in polymers have been proved to be proportional to the compressed chain lengths of molecules [58]. As a result, steric interactions will increase with the length of

polymer chain and effectively protect PDMS surface from attractive interactions that lead to non-specific adsorption, such as electrostatic interactions, van der Waals forces and hydrophobic interactions.

2.3 PAMAM dendrimers as an effective approach to modify PDMS surfaces

Polyamidoamine (PAMAM) dendrimers are monodispersed macromolecules with highly branched three-dimensional structures, as shown in Figure 2-4. Compared with linear polymers, PAMAM dendrimers provide larger area, higher chain flexibility and higher extension to protect the surface from non-specific adsorptions [61, 74].

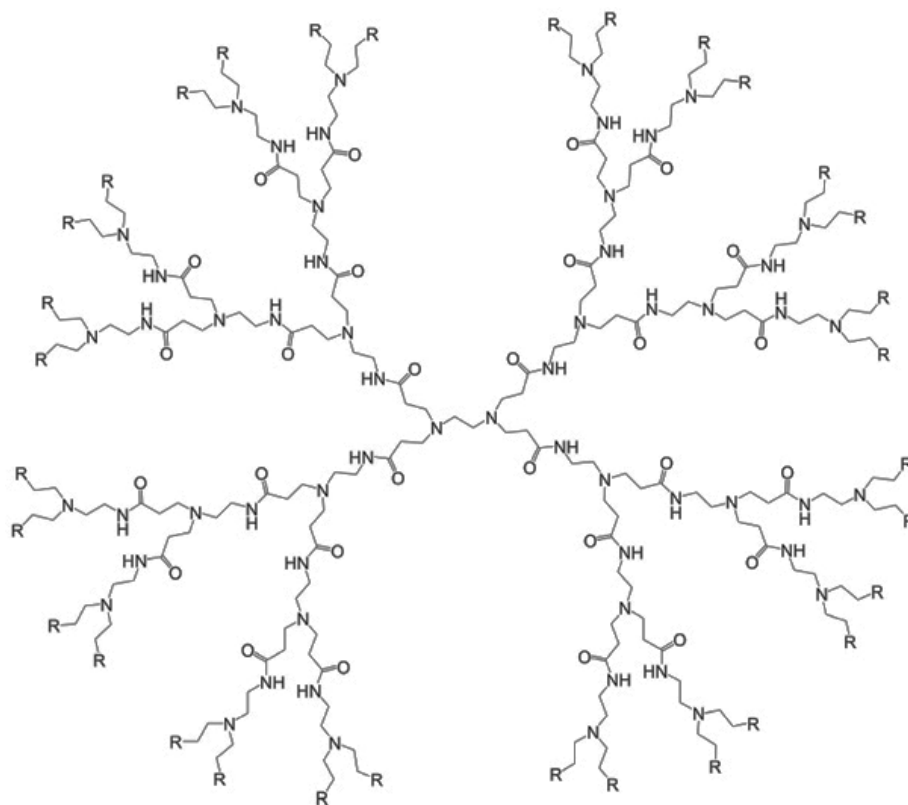


Figure 2-4. Chemical structure of PAMAM dendrimer.

PAMAM dendrimers were first reported that had been synthesized and characterized by Tomalia et al. in early 1980s. Unlike linear polymers, PAMAM dendrimers are highly branched polymers and the growth of PAMAM dendrimers starts from central core material, which is generally propyldiamine, ammonia, ethylenediamine or benzene tricarboxylic acid chloride. The selection of core material is significantly important. This is because the core material will make influences on surface electrification conditions and the formation of chemical structure of PAMAM dendrimers. In traditional synthesis method of PAMAM

dendrimers, the branch-like structures grow from inside of the dendrimers to outside. By using a step-by-step polymerization method, the PAMAM dendrimers were synthesized layer by layer (generation by generation) and finally become highly branched three-dimensional structures, as shown in Figure 2-5 [75]. The synthesis of PAMAM dendrimer includes using methyl acrylate for Michael addition and subsequently using ethylenediamine (EDA) for amidation reactions [75]. In synthesis of PAMAM dendrimers, multistep reactions allow the addition of new generations onto the former generations and the name of the generations are in a continuous number series (e.g. generation 0, generation 1, generation 2). The amino functional groups on the terminals of PAMAM dendrimers can provide positive electric charge to the surface of PAMAM molecules, which can be used in the applications need high density charged surfaces. The generation of PAMAM dendrimer can increase to tenth generation, on which there are 4096 functional sites for one PAMAM dendrimer molecule.

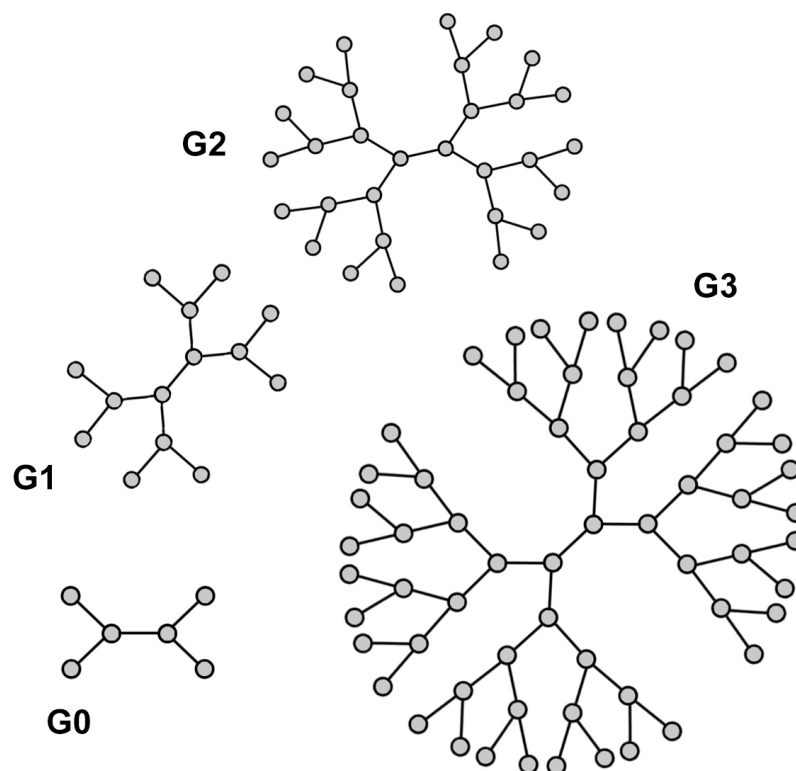


Figure 2-5. Structure of PAMAM dendrimers in different generations.

In addition, Hawker et al. developed a novel convergent growth approach to synthesize PAMAM dendrimers [76-78]. First, branch units are used to produce a dendrite by connecting repetition. Subsequently, core molecule is added to anchor on the former dendrite and contribute the branch units to grow into the multilayer PAMAM dendrimers. The procedures of PAMAM dendrimer synthesis have the possibility to generate small defects within the PAMAM highly branched structures. This is likely caused by intermolecular lactam structure and retro-Michael additions [79]. As a result, the purity of PAMAM dendrimers is generally around 98%.

Recently, PAMAM dendrimers have attracted increasing research interests in many areas, such as biomedicine, surface modification, material science as well as drug delivery system [80-85]. This is because PAMAM dendrimers provide many advantages in these applications. PAMAM dendrimers offer not only large surface-to-volume ratio as a material for surface modification but also provide numerous functional sites on the surface, which can be further modified by specific molecules in different applications such as antibodies and aptamers. Moreover, when the generation of PAMAM dendrimer is greater than 5, the structure of PAMAM dendrimer will demonstrate a spherical, three-dimensional structure. With this structure improvement, the inner structure of PAMAM dendrimer will generate a hydrophobic blank space, which can be utilized as a capsule for various applications [86-90] such as drug delivery systems [88, 91, 92]. PAMAM dendrimer is free immunogenic and nontoxic molecule. This property of PAMAM dendrimers provides the compatibilities of PAMAM dendrimers in biological applications and makes it possible for them to be utilized as markers of tumour tracing and vehicles of drug delivery [80-85]. Meanwhile, PAMAM dendrimers can act as vectors to carry genetic materials into biological systems because of the highly efficient cationic property of PAMAM dendrimers [79, 93-95]. Moreover, PAMAM dendrimers have been used to modify surfaces to prevent non-specific particle adsorptions, since PAMAM dendrimers provide long chain structure with high flexibility and stability and high density of functional groups offered by PAMAM dendrimers can be further modified by hydrophilic functional groups to generate non-fouling surfaces [96, 97]. Li et al.

have developed a strategy to modify PVDF membranes to improve their hydrophilicity and non-fouling performance [97]. In this study, PAMAM dendrimers were used as templates to form silver-PAMAM dendrimer nanocomposites. The nanocomposites provide amine terminated groups and good hydrophilicity and anti-bacterial performance. Scanning electron microscopy (SEM) was used to characterize the modified membranes and results demonstrated that Ag DENPs decreased the pore size and increased the surface roughness of PVDF membranes. Water contact angles were measured to characterize hydrophilicity of the modified surfaces, and results showed that the hydrophilicity of the modified PVDF membranes had been significantly improved.

2.4 Capture ligand and spacer design for CTCs detection

2.4.1 Circulating tumour cells (CTCs)

As it is known to all, cancer is one of the leading causes of death around the world, greatly threaten human's health. It has been reported that more than 90% of cancer deaths result from the development of hematogenously disseminated metastasis [98]. As the most important of rare cells, the presence of circulating tumour cells (CTCs) in patients with cancer was first reported in 1869 [99]. More recently, methods have been developed to detect, isolate and characterize CTCs in multiple different malignancies that arise in solid organs [100]. One of the key pathways to

achieve molecular medicine treatment is through biomarker discovery [101]. During the progression of cancer metastasis, cancer cells shed from solid tumours and enter the bloodstream to become circulating tumour cells [102, 103]. These CTCs have potentials to serve as important biomarkers for early diagnosis of cancer metastases [104, 105]. As a result, sensitive detection of CTCs in the early stage of cancer is significantly important to cancer diagnosis and the finally improvement of the survival rate of cancer patients [104-110].

2.4.2 Antibodies as capture ligands

In the researches of molecular recognition, antibody was the most common biomolecules around the world over last three decades. As a result, many diagnostic assays and inventions based on diagnostic applications in clinics, which are widely used today, were based on the development and contribution of antibodies [111].

Antibodies play a significant role in the field of rare cells detection such as CTCs detection, and diagnosis using diagnostic devices. The applications of antibodies have made significant contributions to the development of applications and technologies of recognition of various molecules. Antibodies were first used to detect analytes in 1950s [112]. In 1970s, antibodies started to be a popular choice in the field of molecular recognition because they could be extracted from the serum of animals. However, the preparation to produce antibodies by polyclonal methods included many complicated steps, which made the production of antibodies could

not meet the demands for them in immunologic studies. In 1975, Kohler and Milstein developed a monoclonal method for antibodies to produce antibodies with large amount [113]. As a result, the method started to be widely used in the field of antibody production around the world [114-116]. In this technology, cell culture methods were used to produce large amount of antibodies with fewer steps in less time, compared with traditional methods of antibody production. In current antibody producing methods, selected monoclonal antibodies can be produced by select clone technology constantly. Theoretically, antibodies can be produced infinitely when assuming the nutriment of cell culture is unlimited. Moreover, immunogens are used in the recognition of monoclonal antibodies. As a result, purification steps for immunogens are not needed, which also can decrease the time in large amount antibody production [117, 118].

The applications of antibodies have made significant contributions to the development of applications and technologies of recognition of various molecules. However, antibodies have also shown their disadvantages [111, 119-121]. First of all, because process of antibody production is taken placed in vivo environment, it is difficult to develop antibodies to that are intensively toxic and weakly immunogenic molecules such as toxins [122]. Because hybridomas are from the process to murine, the applications of antibodies are limited in biological diagnosis and therapy. For example, heterophilic antibodies have possibilities to link with a structure of secondary antibody when there is no recognized molecule, which leads to a false-positive result [123]. In addition, the antibody properties have been proved to be

different in different batches of productions [111]. As a result, people need to characterize the properties of antibodies and experimental conditions in every round of experiments when they used same antibodies but from different batches [124]. Moreover, conditions of in vivo environment are relatively harsh to the process of antibody selection, which makes it difficult for antibody selections to be applied in the environment. Finally, antibodies are sensitive and unstable to temperature, which may result in irreversible thermal denaturation of antibodies. For example, interleukin-1 will undergo an irreversible thermal denaturation process when the temperature of the environment is higher than 54 °C [125, 126].

2.4.3 Aptamers as capture ligands

In recent decades, aptamers have changed the time that antibodies were used as the most popular biomarkers in the applications of diagnostic and therapeutic technologies [111]. Aptamers are single strand oligonucleotides that are selected by systematic evolution of ligands by exponential enrichment (SELEX) from a pool of RNA or DNA library. With the improvement of SELEX technologies, oligonucleotide sequences can be isolated with high purity and the selected aptamers can recognize almost any type of molecules with high specificity and affinity [127-130]. Aptamers are different from antibodies, but aptamers can perform the functions of antibodies with their advantages in most of diagnostic and therapeutic applications [131]. With the development of aptamers technologies, the methods using in different aptamer based applications have been developed in to sophisticated. Many aptamer related

applications have shown the benefits of aptamers with the comparison with antibodies [131-133]. Aptamers can recognize their targets with high specificity and affinity in various ranges of molecule size [134, 135]. In addition, aptamers have shown their excellent performance on recognition of target, molecule separation, analytes detection and clinic diagnosis [136-139]. For example, aptamers can recognize a wide range of analytes from different areas of applications, such as peptides, amino acids, proteins, metal ions and cells [135, 140-142].

Because of high stability, affinity and specificity, aptamers have been used as alternative biomarkers in many fields of studies such as biomarker based affinity purification, bio-imaging and biosensors [140, 141, 143]. Meanwhile, the advantages of aptamers can complement the drawbacks of antibodies, which make the applications of aptamers shown as a promising approach in diagnostic and therapeutic areas. First of all, because the synthesis of aptamers are taken place in a vitro environment instead of in vivo, chemical structures of aptamers can be modified by the requirements of different applications [144]. In addition, aptamers can be used to screen almost any kind of molecules, even molecules with weak response to immunologic systems or with high toxicity [145]. Compared with antibodies, there is little difference of aptamer properties between different batches of productions. This is because aptamers are selected and processed in a standard selection procedure, which makes the production of aptamers possess high repeatability and accuracy [146-149]. Moreover, aptamers can be further modified with molecules at desired points of the DNA sequence without losing their affinities.

The molecules can be fluorescent dyes, functional groups or functional molecules such as biotin for further characterization or modifications in various applications [150-153]. Finally, when modifying nanoparticles with biomarker, the small size of aptamers enables them to be conjugated onto the nanoparticles with a large amount within the limited space on the surface [154-158].

2.4.4 Aptamer-based CTCs detections in microchannels

Because of the advantages talked above, aptamers have been widely used in CTCs detection in microfluidic devices [159-161]. Sheng et al. have developed an aptamer-based microfluidic device for CTCs detection from whole blood [161]. In this study, the microfluidic device was functionalized with aptamers through two-step surface modifications. In the first step, avidin was introduced into the microfluidic device and physically adsorbed onto the glass surface. Subsequently, biotinylated aptamers were immobilized onto the surfaces in microchannel through the reactions between avidin and biotin molecules. The conjugation of aptamers was confirmed via fluorescence microscopy. Finally, target CTCs were introduced into the microchannel and captured by immobilized aptamers through the specific binding cell surface receptors and aptamers. The microfluidic device demonstrated CTCs detections with capture purity around 80% and capture efficiency about 95% [161].

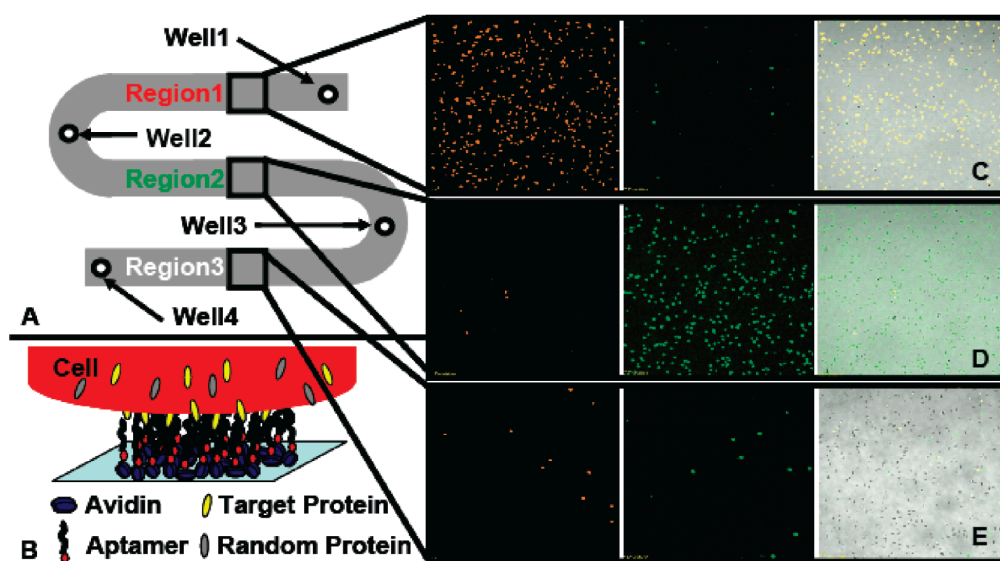


Figure 2-6. Aptamer-based CTCs detection (Reprinted with permission from Ye et al. Copyright 2009 American Chemical Society). Microfluidic device and multiplexed detection of 3 different cancer cell line. (A) Illustration of microfluidic device. Aptamers are immobilized in the three regions and samples are injected from the four wells. (B) Surface structure in microfluidic device, showing avidin adsorbed onto glass surface and biotinylated aptamers are conjugated onto avidin through avidin-biotin interaction. (C-E) Representative image sets at one location at each aptamer-modified region, showing red fluorescent images, green fluorescent images, and double overlays of the fluorescent channels. (C) The Sgc8 aptamer immobilized in Region 1 specifically enriches the red-stained CEM cells. (D) The TD05 aptamer immobilized in Region 2 specifically enriches the green-stained Ramos cells. (E) The Sgd5 aptamer immobilized in Region 3 specifically enriches the unstained Toledo cells.

In addition, Xu et al. have reported a design of microfluidic device for detection of multiple cancer cells [159]. As shown in Figure 2-6, in this study, four wells have been used as inlets and outlets of the microfluidic device to modify three regions of surface in the microchannel. Three different aptamers, Sgc8 aptamer, TD05 aptamer and Sgd5 aptamer were used to modify Region 1, 2 and 3, respectively. It should be noted that aptamer Sgc8, TD05 and Sgd5 are specific aptamers for CEM, Ramos and Toledo cells, respectively. Results of this study showed that the designed microfluidic device could simultaneously sort, enrich and then detect multiple types of cancer cells from a complex sample [159].

2.4.5 Spacers for aptamers

As the beneficial properties aptamers provided, aptamers have been widely used to detect target molecules as biomarkers [162, 163], functionalize liposomes [164], induce micelle formation [165], increase association with cellular membranes [166], increase circulation lifetime [167] and reduce nuclease digestion [168]. The combinations between aptamers and their targets are based on the secondary structure of aptamers [136, 169, 170]. As a result, generally spacers are needed to provide extra space for aptamers to form their specific secondary structures. Oligo-T (thymine) spacers and PEG spacers are two typical spacers that commonly used for aptamers [171]. It should be noted that the modifications of spacers to aptamers may make potential influences on the affinity of aptamers through alteration of the secondary structure of aptamers or steric repulsion, especially the lengths of

spacers [171, 172]. As a result, the investigation of the relationship between aptamers and their spacers is needed in order to provide appropriate environment for aptamers to form their secondary structures.

Brett et al. studied the influences made by different spacers of aptamers on their self-assembly, structure and binding properties [172]. In this study, the aptamers were synthesised with no spacer, oligonucleotide (A_{10} : 10 adenine, T_{10} and T_5 : 10 and 5 thymine) spacers, alkyl (C_{24} and C_{12}) spacers, or polyethylene glycol (PEG_{24} , PEG_8 , PEG_4) spacers. The results showed that the hydrophobic alkyl spacers lost most affinity, hydrophilic PEG spacers enhanced the affinity of the spacers but did not restore it to that of free aptamers. In addition, when aptamers linked with oligonucleotide spacers, the aptamers showed the highest affinity among the three kinds of spacers. A_{10} and T_{10} spacers had equal affinity indicating that nucleotide composition did not affect the affinities of the aptamers. Moreover, Xing et al. investigated how the length of spacers affecting the affinity of aptamers with the existence of PEG modified on liposome surface. In this study, both of PEG molecules and aptamers were modified onto the surface of liposome and results showed that spacers of appropriate length were important to overcome the shielding from surrounding PEG molecules in order to achieve the best targeting effect [171].

2.5 References

- [1] H.-S. Moon, K. Kwon, S.-I. Kim, H. Han, J. Sohn, S. Lee, H.-I. Jung, Continuous separation of breast cancer cells from blood samples using multi-orifice flow fractionation (MOFF) and dielectrophoresis (DEP), *Lab on a Chip*, 11 (2011) 1118-1125.
- [2] S. Bhattacharya, A. Datta, J.M. Berg, S. Gangopadhyay, Studies on surface wettability of poly (dimethyl) siloxane (PDMS) and glass under oxygen-plasma treatment and correlation with bond strength, *Journal of microelectromechanical systems*, 14 (2005) 590-597.
- [3] W. Liu, X. Zhou, D. Xing, Rapid and reliable microRNA detection by stacking hybridization on electrochemiluminescent chip system, *Biosensors and Bioelectronics*, 58 (2014) 388-394.
- [4] C.-F. Wan, C.-S. Chen, K.-C. Hwang, Y.-H. Lai, J.-Y. Luo, P.-C. Liu, L.-S. Fan, Development and automation of microelectromechanical systems-based biochip platform for protein assay, *Sensors and Actuators B: Chemical*, 193 (2014) 53-61.
- [5] J. Horak, C. Dincer, H. Bakirci, G. Urban, Sensitive, rapid and quantitative detection of substance P in serum samples using an integrated microfluidic immunochip, *Biosensors and Bioelectronics*, 58 (2014) 186-192.
- [6] J.-W. Choi, K.W. Oh, J.H. Thomas, W.R. Heineman, H.B. Halsall, J.H. Nevin, A.J. Helmicki, H.T. Henderson, C.H. Ahn, An integrated microfluidic biochemical detection system for protein analysis with magnetic bead-based sampling capabilities, *Lab on a Chip*, 2 (2002) 27-30.
- [7] P.S. Dittrich, P. Schuille, An integrated microfluidic system for reaction, high-sensitivity detection, and sorting of fluorescent cells and particles, *Analytical chemistry*, 75 (2003) 5767-5774.
- [8] K.R. Ackermann, T. Henkel, J. Popp, Quantitative Online Detection of Low - Concentrated Drugs via a SERS Microfluidic System, *ChemPhysChem*, 8 (2007) 2665-2670.
- [9] C.-H. Wang, K.-Y. Lien, J.-J. Wu, G.-B. Lee, A magnetic bead-based assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* by using a microfluidic system with integrated loop-mediated isothermal amplification, *Lab on a Chip*, 11 (2011) 1521-1531.
- [10] A. Vasudev, A. Kaushik, Y. Tomizawa, N. Norena, S. Bhansali, An LTCC-based microfluidic system for label-free, electrochemical detection of cortisol, *Sensors and Actuators B: Chemical*, 182 (2013) 139-146.
- [11] W.-H. Chang, S.-Y. Yang, C.-H. Wang, M.-A. Tsai, P.-C. Wang, T.-Y. Chen, S.-C. Chen, G.-B. Lee, Rapid isolation and detection of aquaculture pathogens in an integrated microfluidic system using loop-mediated isothermal amplification, *Sensors and Actuators B: Chemical*, 180 (2013) 96-106.
- [12] S. Oh, Thick single-layer positive photoresist mold and poly (dimethylsiloxane)(PDMS) dry etching for the fabrication of a glass-PDMS-glass

- microfluidic device, *Journal of Micromechanics and Microengineering*, 18 (2008) 115025.
- [13] P.N. Nge, C.I. Rogers, A.T. Woolley, Advances in microfluidic materials, functions, integration, and applications, *Chemical reviews*, 113 (2013) 2550-2583.
- [14] Q. Tu, J.-C. Wang, Y. Zhang, R. Liu, W. Liu, L. Ren, S. Shen, J. Xu, L. Zhao, J. Wang, Surface modification of poly (dimethylsiloxane) and its applications in microfluidics-based biological analysis, (2012).
- [15] C. Seguin, J.M. McLachlan, P.R. Norton, F. Lagugné-Labarthet, Surface modification of poly (dimethylsiloxane) for microfluidic assay applications, *Applied Surface Science*, 256 (2010) 2524-2531.
- [16] A. Khademhosseini, R. Langer, J. Borenstein, J.P. Vacanti, Microscale technologies for tissue engineering and biology, *Proceedings of the National Academy of Sciences of the United States of America*, 103 (2006) 2480-2487.
- [17] Y.S. Shin, K. Cho, S.H. Lim, S. Chung, S.-J. Park, C. Chung, D.-C. Han, J.K. Chang, PDMS-based micro PCR chip with parylene coating, *Journal of Micromechanics and Microengineering*, 13 (2003) 768.
- [18] T. Fujii, PDMS-based microfluidic devices for biomedical applications, *Microelectronic Engineering*, 61 (2002) 907-914.
- [19] T. Park, S. Lee, G.H. Seong, J. Choo, E.K. Lee, Y.S. Kim, W.H. Ji, S.Y. Hwang, D.-G. Gweon, S. Lee, Highly sensitive signal detection of duplex dye-labelled DNA oligonucleotides in a PDMS microfluidic chip: confocal surface-enhanced Raman spectroscopic study, *Lab on a Chip*, 5 (2005) 437-442.
- [20] X. Weng, C.H. Chon, H. Jiang, D. Li, Rapid detection of formaldehyde concentration in food on a polydimethylsiloxane (PDMS) microfluidic chip, *Food Chemistry*, 114 (2009) 1079-1082.
- [21] G.T. Roman, C.T. Culbertson, Surface engineering of poly (dimethylsiloxane) microfluidic devices using transition metal sol- gel chemistry, *Langmuir*, 22 (2006) 4445-4451.
- [22] M.D. Levenson, N. Viswanathan, R.A. Simpson, Improving resolution in photolithography with a phase-shifting mask, *IEEE Transactions on electron devices*, 29 (1982) 1828-1836.
- [23] P.-Y. Yeh, N.A. Rossi, J.N. Kizhakkedathu, M. Chiao, A silicone-based microfluidic chip grafted with carboxyl functionalized hyperbranched polyglycerols for selective protein capture, *Microfluidics and Nanofluidics*, 9 (2010) 199-209.
- [24] B. Bilenberg, T. Nielsen, B. Clausen, A. Kristensen, PMMA to SU-8 bonding for polymer based lab-on-a-chip systems with integrated optics, *Journal of Micromechanics and Microengineering*, 14 (2004) 814.
- [25] C. Robin, A. Vishnoi, K.N. Jonnalagadda, Mechanical behavior and anisotropy of spin-coated SU-8 thin films for MEMS, *Journal of Microelectromechanical Systems*, 23 (2014) 168-180.
- [26] F. Walther, P. Davydovskaya, S. Zürcher, M. Kaiser, H. Herberg, A.M. Gigler, R.W. Stark, Stability of the hydrophilic behavior of oxygen plasma activated SU-8, *Journal of Micromechanics and Microengineering*, 17 (2007) 524.
- [27] C.S. Sharma, A. Sharma, M. Madou, Multiscale carbon structures fabricated by direct micropatterning of electrospun mats of SU-8 photoresist nanofibers, *Langmuir*, 26 (2010) 2218-2222.

- [28] H. Kotari, M. Motosuke, S. Honami, Particle sorting by optical radiation pressure with low energy density, *La Houille Blanche*, (2013) 72-78.
- [29] E. Zeller, A. Mitchell, Sealed air-core planar waveguide arrays in SU8 epoxy, *Micro-and Nanotechnology: Materials, Processes, Packaging, and Systems IV*, International Society for Optics and Photonics, 2009, pp. 72690E.
- [30] B.R. Watts, Z. Zhang, C.Q. Xu, X. Cao, M. Lin, Scattering detection using a photonic - microfluidic integrated device with on - chip collection capabilities, *Electrophoresis*, 35 (2014) 271-281.
- [31] M.S. Cheri, H. Latifi, J. Sadeghi, M.S. Moghaddam, H. Shahraki, H. Hajghassem, Real-time measurement of flow rate in microfluidic devices using a cantilever-based optofluidic sensor, *Analyst*, 139 (2014) 431-438.
- [32] F. Perdigones, J.M. García, J.M. Quero, Fabrication process for integration of SU-8 check valves on printed circuit board-based microfluidic platforms, *Micro & Nano Letters*, 9 (2014) 37-40.
- [33] Z. Zhang, P. Zhao, G. Xiao, B.R. Watts, C. Xu, Sealing SU-8 microfluidic channels using PDMS, *Biomicrofluidics*, 5 (2011) 046503.
- [34] C.-W. Tsao, D.L. DeVoe, Bonding of thermoplastic polymer microfluidics, *Microfluidics and Nanofluidics*, 6 (2009) 1-16.
- [35] H. Hillborg, J. Ankner, U.W. Gedde, G. Smith, H. Yasuda, K. Wikström, Crosslinked polydimethylsiloxane exposed to oxygen plasma studied by neutron reflectometry and other surface specific techniques, *Polymer*, 41 (2000) 6851-6863.
- [36] P.Y. Yeh, Z. Zhang, M. Lin, X. Cao, Nonfouling hydrophilic poly (ethylene glycol) engraftment strategy for PDMS/SU-8 heterogeneous microfluidic devices, *Langmuir*, 28 (2012) 16227-16236.
- [37] S.H. Tan, N.-T. Nguyen, Y.C. Chua, T.G. Kang, Oxygen plasma treatment for reducing hydrophobicity of a sealed polydimethylsiloxane microchannel, *Biomicrofluidics*, 4 (2010) 032204.
- [38] Y.J. Kim, I.-K. Kang, M.W. Huh, S.-C. Yoon, Surface characterization and in vitro blood compatibility of poly (ethylene terephthalate) immobilized with insulin and/or heparin using plasma glow discharge, *Biomaterials*, 21 (2000) 121-130.
- [39] J.S. Kong, D.J. Lee, H.D. Kim, Surface modification of low - density polyethylene (LDPE) film and improvement of adhesion between evaporated copper metal film and LDPE, *Journal of applied polymer science*, 82 (2001) 1677-1690.
- [40] A. Oyane, M. Uchida, Y. Yokoyama, C. Choong, J. Triffitt, A. Ito, Simple surface modification of poly (ϵ - caprolactone) to induce its apatite - forming ability, *Journal of Biomedical Materials Research Part A*, 75 (2005) 138-145.
- [41] M.J. Wang, Y.I. Chang, F. Poncin - Epailard, Acid and basic functionalities of nitrogen and carbon dioxide plasma - treated polystyrene, *Surface and interface analysis*, 37 (2005) 348-355.
- [42] Y. Hu, S.R. Winn, I. Krajchich, J.O. Hollinger, Porous polymer scaffolds surface - modified with arginine - glycine - aspartic acid enhance bone cell attachment and differentiation in vitro, *Journal of Biomedical Materials Research Part A*, 64 (2003) 583-590.

- [43] M. Crombez, P. Chevallier, E. Petitclerc, D. Mantovani, G. Laroche, Improving arterial prosthesis neo-endothelialization: application of a proactive VEGF construct onto PTFE surfaces, *Biomaterials*, 26 (2005) 7402-7409.
- [44] P. Chevallier, R. Janvier, D. Mantovani, G. Laroche, In vitro Biological Performances of Phosphorylcholine - Grafted ePTFE Prostheses through RFGD Plasma Techniques, *Macromolecular bioscience*, 5 (2005) 829-839.
- [45] M. Aouinti, P. Bertrand, F. Poncin-Epaillard, Characterization of polypropylene surface treated in a CO₂ plasma, *Plasmas and polymers*, 8 (2003) 225-236.
- [46] J.G. Terlingen, H.F. Gerritsen, A.S. Hoffman, J. Feijen, Introduction of functional groups on polyethylene surfaces by a carbon dioxide plasma treatment, *Journal of applied polymer science*, 57 (1995) 969-982.
- [47] N. Medard, J.-C. Soutif, F. Poncin-Epaillard, Characterization of CO₂ plasma-treated polyethylene surface bearing carboxylic groups, *Surface and Coatings Technology*, 160 (2002) 197-205.
- [48] V. Vasilets, G. Hermel, U. König, C. Werner, M. Müller, F. Simon, K. Grundke, Y. Ikada, H.-J. Jacobasch, Microwave CO₂ plasma-initiated vapour phase graft polymerization of acrylic acid onto polytetrafluoroethylene for immobilization of human thrombomodulin, *Biomaterials*, 18 (1997) 1139-1145.
- [49] L. Brown, T. Koerner, J.H. Horton, R.D. Oleschuk, Fabrication and characterization of poly (methylmethacrylate) microfluidic devices bonded using surface modifications and solvents, *Lab on a Chip*, 6 (2006) 66-73.
- [50] D. Rymuszka, K. Terpilowski, L. Holysz, E. Chibowski, Changes in surface properties of polymethylmethacrylate (PMMA) treated with air plasma, *Annales Universitatis Mariae Curie-Sklodowska, De Gruyter Open Sp. z oo*, 2015, pp. 65.
- [51] Z. Cheng, S.-H. Teoh, Surface modification of ultra thin poly (ϵ -caprolactone) films using acrylic acid and collagen, *Biomaterials*, 25 (2004) 1991-2001.
- [52] Z. Ademovic, D. Klee, P. Kingshott, R. Kaufmann, H. Höcker, Minimization of protein adsorption on poly (vinylidene fluoride), *Biomolecular engineering*, 19 (2002) 177-182.
- [53] E. Kang, K. Tan, K. Kato, Y. Uyama, Y. Ikada, Surface modification and functionalization of polytetrafluoroethylene films, *Macromolecules*, 29 (1996) 6872-6879.
- [54] L. Cen, K. Neoh, L. Ying, E. Kang, Surface modification of polymeric films and membranes to achieve antibacterial properties, *Surface and interface analysis*, 36 (2004) 716-719.
- [55] P. Wang, K. Tan, E. Kang, K. Neoh, Surface functionalization of low density polyethylene films with grafted poly (ethylene glycol) derivatives, *Journal of Materials Chemistry*, 11 (2001) 2951-2957.
- [56] C. Séguin, J.M. McLachlan, P.R. Norton, F. Lagurné-Labarthe, Surface modification of poly (dimethylsiloxane) for microfluidic assay applications, *Applied Surface Science*, 256 (2010) 2524-2531.
- [57] Z. Zhang, P. Zhao, G. Xiao, The fabrication of polymer microfluidic devices using a solid-to-solid interfacial polyaddition, *Polymer*, 50 (2009) 5358-5361.

- [58] P.-Y. Yeh, J.N. Kizhakkedathu, M. Chiao, A novel method to attenuate protein adsorption using combinations of polyethylene glycol (PEG) grafts and piezoelectric actuation, *Journal of Nanotechnology in Engineering and Medicine*, 1 (2010) 041010.
- [59] C.-C. Wu, C.-Y. Yuan, S.-J. Ding, Effect of polydimethylsiloxane surfaces silanized with different nitrogen-containing groups on the adhesion progress of epithelial cells, *Surface and Coatings Technology*, 205 (2011) 3182-3189.
- [60] J.N. Lee, X. Jiang, D. Ryan, G.M. Whitesides, Compatibility of mammalian cells on surfaces of poly (dimethylsiloxane), *Langmuir*, 20 (2004) 11684-11691.
- [61] P. Kingshott, H. Thissen, H.J. Griesser, Effects of cloud-point grafting, chain length, and density of PEG layers on competitive adsorption of ocular proteins, *Biomaterials*, 23 (2002) 2043-2056.
- [62] J.A. Vickers, M.M. Caulum, C.S. Henry, Generation of hydrophilic poly (dimethylsiloxane) for high-performance microchip electrophoresis, *Analytical Chemistry*, 78 (2006) 7446-7452.
- [63] Y. Liu, J.C. Fanguy, J.M. Bledsoe, C.S. Henry, Dynamic coating using polyelectrolyte multilayers for chemical control of electroosmotic flow in capillary electrophoresis microchips, *Analytical chemistry*, 72 (2000) 5939-5944.
- [64] K.W. Ro, W.J. Chang, H. Kim, Y.M. Koo, J.H. Hahn, Capillary electrochromatography and preconcentration of neutral compounds on poly (dimethylsiloxane) microchips, *Electrophoresis*, 24 (2003) 3253-3259.
- [65] D. Xiao, H. Zhang, M. Wirth, Chemical modification of the surface of poly (dimethylsiloxane) by atom-transfer radical polymerization of acrylamide, *Langmuir*, 18 (2002) 9971-9976.
- [66] S.H. Kim, Y. Yang, M. Kim, S.W. Nam, K.M. Lee, N.Y. Lee, Y.S. Kim, S. Park, Simple Route to Hydrophilic Microfluidic Chip Fabrication Using an Ultraviolet (UV) - Cured Polymer, *Advanced Functional Materials*, 17 (2007) 3493-3498.
- [67] T. Wu, K. Efimenko, J. Genzer, Combinatorial study of the mushroom-to-brush crossover in surface anchored polyacrylamide, *Journal of the american chemical society*, 124 (2002) 9394-9395.
- [68] S. Tugulu, H.A. Klok, Surface modification of polydimethylsiloxane substrates with nonfouling poly (poly (ethylene glycol) methacrylate) brushes, *Macromolecular symposia*, Wiley Online Library, 2009, pp. 103-109.
- [69] H. Sasaki, H. Onoe, T. Osaki, R. Kawano, S. Takeuchi, Parylene-coating in PDMS microfluidic channels prevents the absorption of fluorescent dyes, *Sensors and Actuators B: Chemical*, 150 (2010) 478-482.
- [70] J.S. Swensen, Y. Xiao, B.S. Ferguson, A.A. Lubin, R.Y. Lai, A.J. Heeger, K.W. Plaxco, H.T. Soh, Continuous, real-time monitoring of cocaine in undiluted blood serum via a microfluidic, electrochemical aptamer-based sensor, *Journal of the American Chemical Society*, 131 (2009) 4262-4266.
- [71] M.A. Hahn, P.C. Keng, T.D. Krauss, Flow cytometric analysis to detect pathogens in bacterial cell mixtures using semiconductor quantum dots, *Analytical chemistry*, 80 (2008) 864-872.
- [72] Z. Zhang, X. Feng, F. Xu, X. Liu, B.F. Liu, "Click" chemistry - based surface modification of poly (dimethylsiloxane) for protein separation in a microfluidic chip, *Electrophoresis*, 31 (2010) 3129-3136.

- [73] Z. Zhang, X. Feng, Q. Luo, B.F. Liu, Environmentally friendly surface modification of PDMS using PEG polymer brush, *Electrophoresis*, 30 (2009) 3174-3180.
- [74] P.-Y.J. Yeh, R.K. Kainthan, Y. Zou, M. Chiao, J.N. Kizhakkedathu, Self-assembled monothiol-terminated hyperbranched polyglycerols on a gold surface: a comparative study on the structure, morphology, and protein adsorption characteristics with linear poly (ethylene glycol) s, *Langmuir*, 24 (2008) 4907-4916.
- [75] D.A. Tomalia, A.M. Naylor, W.A. Goddard, Starburst dendrimers: Molecular - level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter, *Angewandte Chemie International Edition*, 29 (1990) 138-175.
- [76] C.J. Hawker, J.M. Frechet, Preparation of polymers with controlled molecular architecture. A new convergent approach to dendritic macromolecules, *Journal of the American Chemical Society*, 112 (1990) 7638-7647.
- [77] G. Newkome, C. Moorefield, F. Vögtle, Synthetic methodologies: convergent procedures, *Dendritic Molecules: Concepts, Syntheses, Perspectives*, (1996) 107-163.
- [78] K.L. Wooley, C.J. Hawker, J.M. Fréchet, Polymers with controlled molecular architecture: control of surface functionality in the synthesis of dendritic hyperbranched macromolecules using the convergent approach, *Journal of the Chemical Society, Perkin Transactions 1*, (1991) 1059-1076.
- [79] J.D. Eichman, A.U. Bielinska, J.F. Kukowska-Latallo, J.R. Baker, The use of PAMAM dendrimers in the efficient transfer of genetic material into cells, *Pharmaceutical science & technology today*, 3 (2000) 232-245.
- [80] B.K. Nanjwade, H.M. Bechra, G.K. Derkar, F. Manvi, V.K. Nanjwade, Dendrimers: emerging polymers for drug-delivery systems, *European Journal of Pharmaceutical Sciences*, 38 (2009) 185-196.
- [81] K. Madaan, S. Kumar, N. Poonia, V. Lather, D. Pandita, Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues, *Journal of pharmacy & bioallied sciences*, 6 (2014) 139.
- [82] E.R. Gillies, J.M. Frechet, Dendrimers and dendritic polymers in drug delivery, *Drug discovery today*, 10 (2005) 35-43.
- [83] A.K. Patri, J.F. Kukowska-Latallo, J.R. Baker, Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex, *Advanced drug delivery reviews*, 57 (2005) 2203-2214.
- [84] A. D'emanuele, D. Attwood, Dendrimer-drug interactions, *Advanced drug delivery reviews*, 57 (2005) 2147-2162.
- [85] Y.E. Kurtoglu, M.K. Mishra, S. Kannan, R.M. Kannan, Drug release characteristics of PAMAM dendrimer-drug conjugates with different linkers, *International journal of pharmaceutics*, 384 (2010) 189-194.
- [86] A.K. Patri, I.J. Majoros, J.R. Baker, Dendritic polymer macromolecular carriers for drug delivery, *Current opinion in chemical biology*, 6 (2002) 466-471.
- [87] H.R. Ihre, O.L. Padilla De Jesús, F.C. Szoka, J.M. Fréchet, Polyester dendritic systems for drug delivery applications: design, synthesis, and characterization, *Bioconjugate chemistry*, 13 (2002) 443-452.

- [88] R. Esfand, D.A. Tomalia, Poly (amidoamine)(PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications, *Drug discovery today*, 6 (2001) 427-436.
- [89] O.V. Lebedeva, B.-S. Kim, F. Gröhn, O.I. Vinogradova, Dendrimer-encapsulated gold nanoparticles as building blocks for multilayer microshells, *Polymer*, 48 (2007) 5024-5029.
- [90] H. Wu, Z. Liu, X. Wang, B. Zhao, J. Zhang, C. Li, Preparation of hollow capsule-stabilized gold nanoparticles through the encapsulation of the dendrimer, *Journal of colloid and interface science*, 302 (2006) 142-148.
- [91] M. Liu, J.M. Fréchet, Designing dendrimers for drug delivery, *Pharmaceutical science & technology today*, 2 (1999) 393-401.
- [92] C.C. Lee, J.A. MacKay, J.M. Fréchet, F.C. Szoka, Designing dendrimers for biological applications, *Nature biotechnology*, 23 (2005) 1517-1526.
- [93] J.L. Santos, H. Oliveira, D. Pandita, J. Rodrigues, A.P. Pêgo, P.L. Granja, H. Tomás, Functionalization of poly (amidoamine) dendrimers with hydrophobic chains for improved gene delivery in mesenchymal stem cells, *Journal of Controlled Release*, 144 (2010) 55-64.
- [94] M.X. Tang, C.T. Redemann, F.C. Szoka, In vitro gene delivery by degraded polyamidoamine dendrimers, *Bioconjugate chemistry*, 7 (1996) 703-714.
- [95] C. Dufes, I.F. Uchegbu, A.G. Schätzlein, Dendrimers in gene delivery, *Advanced drug delivery reviews*, 57 (2005) 2177-2202.
- [96] D. Astruc, E. Boisselier, C. Ornelas, Dendrimers designed for functions: from physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine, *Chemical reviews*, 110 (2010) 1857-1959.
- [97] G. Li, L. Shen, Y. Luo, S. Zhang, The effect of silver-PAMAM dendrimer nanocomposites on the performance of PVDF membranes, *Desalination*, 338 (2014) 115-120.
- [98] M.S. Wicha, D.F. Hayes, Circulating tumor cells: not all detected cells are bad and not all bad cells are detected, *American Society of Clinical Oncology*, 2011.
- [99] M.C. Miller, G.V. Doyle, L.W. Terstappen, Significance of circulating tumor cells detected by the CellSearch system in patients with metastatic breast colorectal and prostate cancer, *Journal of oncology*, 2010 (2009).
- [100] D.F. Hayes, J. Smerage, Is there a role for circulating tumor cells in the management of breast cancer?, *Clinical Cancer Research*, 14 (2008) 3646-3650.
- [101] W. Tan, M.J. Donovan, J. Jiang, Aptamers from cell-based selection for bioanalytical applications, *Chemical reviews*, 113 (2013) 2842-2862.
- [102] P.S. Steeg, Tumor metastasis: mechanistic insights and clinical challenges, *Nature medicine*, 12 (2006) 895.
- [103] G.P. Gupta, J. Massagué, Cancer metastasis: building a framework, *Cell*, 127 (2006) 679-695.
- [104] M. Cristofanilli, G.T. Budd, M.J. Ellis, A. Stopeck, J. Matera, M.C. Miller, J.M. Reuben, G.V. Doyle, W.J. Allard, L.W. Terstappen, Circulating tumor cells, disease progression, and survival in metastatic breast cancer, *New England Journal of Medicine*, 351 (2004) 781-791.

- [105] D.C. Danila, M. Fleisher, H.I. Scher, Circulating tumor cells as biomarkers in prostate cancer, *Clinical Cancer Research*, 17 (2011) 3903-3912.
- [106] P. Paterlini-Brechot, N.L. Benali, Circulating tumor cells (CTC) detection: clinical impact and future directions, *Cancer letters*, 253 (2007) 180-204.
- [107] E.S. Lianidou, A. Markou, Circulating tumor cells in breast cancer: detection systems, molecular characterization, and future challenges, *Clinical chemistry*, 57 (2011) 1242-1255.
- [108] S. Riethdorf, H. Fritsche, V. Müller, T. Rau, C. Schindlbeck, B. Rack, W. Janni, C. Coith, K. Beck, F. Jänicke, Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system, *Clinical cancer research*, 13 (2007) 920-928.
- [109] X. Wang, X. Qian, J.J. Beitler, Z.G. Chen, F.R. Khuri, M.M. Lewis, H.J.C. Shin, S. Nie, D.M. Shin, Detection of circulating tumor cells in human peripheral blood using surface-enhanced Raman scattering nanoparticles, *Cancer research*, 71 (2011) 1526-1532.
- [110] F. Fabbri, S. Carloni, W. Zoli, P. Ulivi, G. Gallerani, P. Fici, E. Chiadini, A. Passardi, G.L. Frassineti, A. Ragazzini, Detection and recovery of circulating colon cancer cells using a dielectrophoresis-based device: KRAS mutation status in pure CTCs, *Cancer letters*, 335 (2013) 225-231.
- [111] S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, *Clinical chemistry*, 45 (1999) 1628-1650.
- [112] R.S. Yalow, S.A. Berson, Assay of plasma insulin in human subjects by immunological methods, *Nature*, 184 (1959) 1648-1649.
- [113] G. Köhler, C. Milstein, Continuous cultures of fused cells secreting antibody of predefined specificity, *nature*, 256 (1975) 495-497.
- [114] H.G. Gratzner, Monoclonal antibody to 5-bromo-and 5-iododeoxyuridine: a new reagent for detection of DNA replication, *Science*, 218 (1982) 474-475.
- [115] G. Köhler, C. Milstein, Derivation of specific antibody - producing tissue culture and tumor lines by cell fusion, *European journal of immunology*, 6 (1976) 511-519.
- [116] R. Hawkes, E. Niday, J. Gordon, A dot-immunobinding assay for monoclonal and other antibodies, *Analytical biochemistry*, 119 (1982) 142-147.
- [117] J.P. Welsh, M.G. Petroff, P. Rowicki, H. Bao, T. Linden, D.J. Roush, J.M. Pollard, A practical strategy for using miniature chromatography columns in a standardized high - throughput workflow for purification development of monoclonal antibodies, *Biotechnology progress*, 30 (2014) 626-635.
- [118] M.F. Silva, A. Fernandes-Platzgummer, M.R. Aires-Barros, A.M. Azevedo, Integrated purification of monoclonal antibodies directly from cell culture medium with aqueous two-phase systems, *Separation and Purification Technology*, 132 (2014) 330-335.
- [119] Y. Xia, M. Liu, L. Wang, A. Yan, W. He, M. Chen, J. Lan, J. Xu, L. Guan, J. Chen, A visible and colorimetric aptasensor based on DNA-capped single-walled carbon nanotubes for detection of exosomes, *Biosensors and Bioelectronics*, 92 (2017) 8-15.

- [120] M.-M. Zhan, X.-Q. Hu, X.-X. Liu, B.-F. Ruan, J. Xu, C. Liao, From monoclonal antibodies to small molecules: the development of inhibitors targeting the PD-1/PD-L1 pathway, *Drug discovery today*, 21 (2016) 1027-1036.
- [121] F. Lin, Z. Li, Y. Hua, Y.P. Lim, Proteomic profiling predicts drug response to novel targeted anticancer therapeutics, *Expert review of proteomics*, 13 (2016) 411-420.
- [122] J. Van Ness, U.K. Laemmli, D.E. Pettijohn, Immunization in vitro and production of monoclonal antibodies specific to insoluble and weakly immunogenic proteins, *Proceedings of the National Academy of Sciences*, 81 (1984) 7897-7901.
- [123] D. Cook, C. Self, Monoclonal antibodies in diagnostic immunoassays, *Monoclonal antibodies*, (1995) 180-208.
- [124] M. Schwarz, L. Spector, A. Gargir, A. Shtevi, M. Gortler, R.T. Altstock, A.A. Dukler, N. Dotan, A new kind of carbohydrate array, its use for profiling antiglycan antibodies, and the discovery of a novel human cellulose-binding antibody, *Glycobiology*, 13 (2003) 749-754.
- [125] T.F. Linsenmayer, J.M. Fitch, T.M. Schmid, E. Gibney, R. Sanderson, R. Mayne, Monoclonal antibodies against chicken type V collagen: production, specificity, and use for immunocytochemical localization in embryonic cornea and other organs, *The Journal of cell biology*, 96 (1983) 124-132.
- [126] L.L. Chang, D. Shepherd, J. Sun, X.C. Tang, M.J. Pikal, Effect of sorbitol and residual moisture on the stability of lyophilized antibodies: Implications for the mechanism of protein stabilization in the solid state, *Journal of pharmaceutical sciences*, 94 (2005) 1445-1455.
- [127] J. Zhou, M.R. Battig, Y. Wang, Aptamer-based molecular recognition for biosensor development, *Analytical and bioanalytical chemistry*, 398 (2010) 2471-2480.
- [128] X. Fang, W. Tan, Aptamers generated from cell-SELEX for molecular medicine: a chemical biology approach, *Accounts of chemical research*, 43 (2009) 48-57.
- [129] T. Mairal, V.C. Özalp, P.L. Sánchez, M. Mir, I. Katakis, C.K. O'Sullivan, Aptamers: molecular tools for analytical applications, *Analytical and bioanalytical chemistry*, 390 (2008) 989-1007.
- [130] K. Sefah, D. Shangguan, X. Xiong, M.B. O'donoghue, W. Tan, Development of DNA aptamers using Cell-SELEX, *Nature protocols*, 5 (2010) 1169-1185.
- [131] Y. Xu, X. Yang, E. Wang, Review: Aptamers in microfluidic chips, *Analytica chimica acta*, 683 (2010) 12-20.
- [132] U. Schlecht, A. Malave, T. Gronewold, M. Tewes, M. Löhndorf, Comparison of antibody and aptamer receptors for the specific detection of thrombin with a nanometer gap-sized impedance biosensor, *Analytica chimica acta*, 573 (2006) 65-68.
- [133] S.Y. Toh, M. Citartan, S.C. Gopinath, T.-H. Tang, Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay, *Biosensors and Bioelectronics*, 64 (2015) 392-403.
- [134] G. Hybarger, J. Bynum, R.F. Williams, J.J. Valdes, J.P. Chambers, A microfluidic SELEX prototype, *Analytical and bioanalytical chemistry*, 384 (2006) 191-198.

- [135] X. Lou, J. Qian, Y. Xiao, L. Viel, A.E. Gerdon, E.T. Lagally, P. Atzberger, T.M. Tarasow, A.J. Heeger, H.T. Soh, Micromagnetic selection of aptamers in microfluidic channels, *Proceedings of the National Academy of Sciences*, 106 (2009) 2989-2994.
- [136] T. Hermann, D.J. Patel, Adaptive recognition by nucleic acid aptamers, *Science*, 287 (2000) 820-825.
- [137] X. He, B. Wei, Y. Mi, Aptamer based reversible DNA induced hydrogel system for molecular recognition and separation, *Chemical communications*, 46 (2010) 6308-6310.
- [138] G. Liu, X. Mao, J.A. Phillips, H. Xu, W. Tan, L. Zeng, Aptamer– nanoparticle strip biosensor for sensitive detection of cancer cells, *Analytical chemistry*, 81 (2009) 10013-10018.
- [139] X. Wu, J. Chen, M. Wu, J.X. Zhao, Aptamers: active targeting ligands for cancer diagnosis and therapy, *Theranostics*, 5 (2015) 322.
- [140] R. Kirby, E.J. Cho, B. Gehrke, T. Bayer, Y.S. Park, D.P. Neikirk, J.T. McDevitt, A.D. Ellington, Aptamer-based sensor arrays for the detection and quantitation of proteins, *Analytical Chemistry*, 76 (2004) 4066-4075.
- [141] R.K. Mosing, M.T. Bowser, Microfluidic selection and applications of aptamers, *Journal of separation science*, 30 (2007) 1420-1426.
- [142] W.-I. Wu, K.N. Sask, J.L. Brash, P.R. Selvaganapathy, Polyurethane-based microfluidic devices for blood contacting applications, *Lab on a Chip*, 12 (2012) 960-970.
- [143] W. Xu, Y. Wang, S. Liu, J. Yu, H. Wang, J. Huang, A novel sandwich-type electrochemical aptasensor for sensitive detection of kanamycin based on GR–PANI and PAMAM–Au nanocomposites, *New Journal of Chemistry*, 38 (2014) 4931-4937.
- [144] K.-M. Song, S. Lee, C. Ban, Aptamers and their biological applications, *Sensors*, 12 (2012) 612-631.
- [145] J.G. Walter, F. Stahl, T. Scheper, Aptamers as affinity ligands for downstream processing, *Engineering in Life Sciences*, 12 (2012) 496-506.
- [146] R. Stoltenburg, C. Reinemann, B. Strehlitz, FluMag-SELEX as an advantageous method for DNA aptamer selection, *Analytical and bioanalytical chemistry*, 383 (2005) 83-91.
- [147] D.A. Daniels, H. Chen, B.J. Hicke, K.M. Swiderek, L. Gold, A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment, *Proceedings of the National Academy of Sciences*, 100 (2003) 15416-15421.
- [148] R.K. Mosing, S.D. Mendonsa, M.T. Bowser, Capillary electrophoresis-SELEX selection of aptamers with affinity for HIV-1 reverse transcriptase, *Analytical Chemistry*, 77 (2005) 6107-6112.
- [149] S.C.B. Gopinath, Methods developed for SELEX, *Analytical and bioanalytical chemistry*, 387 (2007) 171-182.
- [150] R. E Wang, Y. Zhang, J. Cai, W. Cai, T. Gao, Aptamer-based fluorescent biosensors, *Current medicinal chemistry*, 18 (2011) 4175-4184.
- [151] J.E. Smith, C.D. Medley, Z. Tang, D. Shangguan, C. Lofton, W. Tan, Aptamer-conjugated nanoparticles for the collection and detection of multiple cancer cells, *Analytical Chemistry*, 79 (2007) 3075-3082.

- [152] S. Song, L. Wang, J. Li, C. Fan, J. Zhao, Aptamer-based biosensors, *TrAC Trends in Analytical Chemistry*, 27 (2008) 108-117.
- [153] J.A. Phillips, D. Lopez-Colon, Z. Zhu, Y. Xu, W. Tan, Applications of aptamers in cancer cell biology, *Analytica chimica acta*, 621 (2008) 101-108.
- [154] C.-C. Huang, Y.-F. Huang, Z. Cao, W. Tan, H.-T. Chang, Aptamer-modified gold nanoparticles for colorimetric determination of platelet-derived growth factors and their receptors, *Analytical chemistry*, 77 (2005) 5735-5741.
- [155] S.-J. Chen, Y.-F. Huang, C.-C. Huang, K.-H. Lee, Z.-H. Lin, H.-T. Chang, Colorimetric determination of urinary adenosine using aptamer-modified gold nanoparticles, *Biosensors and Bioelectronics*, 23 (2008) 1749-1753.
- [156] Y.-F. Huang, Y.-W. Lin, Z.-H. Lin, H.-T. Chang, Aptamer-modified gold nanoparticles for targeting breast cancer cells through light scattering, *Journal of Nanoparticle Research*, 11 (2009) 775-783.
- [157] J.K. Herr, J.E. Smith, C.D. Medley, D. Shangguan, W. Tan, Aptamer-conjugated nanoparticles for selective collection and detection of cancer cells, *Analytical Chemistry*, 78 (2006) 2918-2924.
- [158] C.D. Medley, J.E. Smith, Z. Tang, Y. Wu, S. Bamrungsap, W. Tan, Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells, *Analytical chemistry*, 80 (2008) 1067-1072.
- [159] Y. Xu, J.A. Phillips, J. Yan, Q. Li, Z.H. Fan, W. Tan, Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells, *Analytical chemistry*, 81 (2009) 7436-7442.
- [160] J. Chen, J. Li, Y. Sun, Microfluidic approaches for cancer cell detection, characterization, and separation, *Lab on a Chip*, 12 (2012) 1753-1767.
- [161] W. Sheng, T. Chen, R. Kamath, X. Xiong, W. Tan, Z.H. Fan, Aptamer-enabled efficient isolation of cancer cells from whole blood using a microfluidic device, *Analytical chemistry*, 84 (2012) 4199-4206.
- [162] S. Centi, S. Tombelli, M. Minunni, M. Mascini, Aptamer-based detection of plasma proteins by an electrochemical assay coupled to magnetic beads, *Analytical chemistry*, 79 (2007) 1466-1473.
- [163] Y. Xiao, A.A. Lubin, A.J. Heeger, K.W. Plaxco, Label - free electronic detection of thrombin in blood serum by using an aptamer - based sensor, *Angewandte Chemie*, 117 (2005) 5592-5595.
- [164] Z. Cao, R. Tong, A. Mishra, W. Xu, G.C. Wong, J. Cheng, Y. Lu, Reversible Cell - Specific Drug Delivery with Aptamer - Functionalized Liposomes, *Angewandte Chemie International Edition*, 48 (2009) 6494-6498.
- [165] H. Liu, Z. Zhu, H. Kang, Y. Wu, K. Sefan, W. Tan, DNA - based micelles: synthesis, micellar properties and size - dependent cell permeability, *Chemistry - A European Journal*, 16 (2010) 3791-3797.
- [166] G.G. Borisenko, M.A. Zaitseva, A.N. Chuvilin, G.E. Pozmogova, DNA modification of live cell surface, *Nucleic acids research*, 37 (2009) e28-e28.
- [167] P. Bouchard, R. Hutabarat, K. Thompson, Discovery and development of therapeutic aptamers, *Annual review of pharmacology and toxicology*, 50 (2010) 237-257.

- [168] F. Tolle, G. Mayer, Dressed for success—applying chemistry to modulate aptamer functionality, *Chemical Science*, 4 (2013) 60-67.
- [169] M.N. Stojanovic, P. De Prada, D.W. Landry, Aptamer-based folding fluorescent sensor for cocaine, *Journal of the American Chemical Society*, 123 (2001) 4928-4931.
- [170] F. Jiang, R.A. Kumar, R.A. Jones, D.J. Patel, Structural basis of RNA folding and recognition in an AMP–RNA aptamer complex, *Nature*, 382 (1996) 183-186.
- [171] Y.-H. Lao, K. Peck, L.-C. Chen, Enhancement of aptamer microarray sensitivity through spacer optimization and avidity effect, *Analytical chemistry*, 81 (2009) 1747-1754.
- [172] B. Waybrant, T.R. Pearce, E. Kokkoli, Effect of polyethylene glycol, alkyl, and oligonucleotide spacers on the binding, secondary structure, and self-assembly of fractalkine binding FKN-S2 aptamer-amphiphiles, *Langmuir*, 30 (2014) 7465-7474.

**Chapter 3 Developing an ultra non-fouling SU-8 and PDMS
hybrid microfluidic device by poly(amidoamine) engraftment**

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3.1 Abstract

A new strategy to prepare an ultra non-fouling SU-8 / poly(dimethylsiloxane) (PDMS) hybrid microfluidic system is presented. We report a simple method to bond both SU-8 and PDMS surfaces to prepare the hybrid microfluidic device and then simultaneously modify both SU-8 and PDMS surfaces using poly(amidoamine) (PAMAM), a highly hydrophilic dendrimer to enhance non-fouling properties of the hybrid microfluidic channel. Water contact angle, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) are used to characterize and confirm surface modifications. Finally the non-fouling performance of the resulting microfluidic system is tested using both microbeads and *Escherichia coli* O157:H7 bacterial cells. We demonstrate that the obtained hybrid microfluidic system shows a significant microbead adsorption suppression of 99.7%, an ultra non-fouling performance that has not been reported in the literature before; in addition, 95% bacteria surface adsorption suppression is also obtained. Considering the significantly improved non-fouling performance and the ease of preparing the hybrid microfluidic system, we anticipate that this strategy will find wide applications in biosensing and detection.

3.2 Introduction

The importance of microfluidic systems with applications in biological and chemical analysis has grown significantly in biology, diagnostics and biochemistry [1]. This is because these microfluidic platforms offer many advantages such as low sample volume requirement [2], rapid reaction, high sensitivity [3], low power consumption and relatively low cost to fabricate [4]. Poly(dimethylsiloxane) (PDMS) is a commonly used material to fabricate microfluidic channels because it has many desirable properties, such as optical transparency [5], chemical inertness [6], moldability [7], high elasticity [8], as well as excellent gas permeability to O_2 and CO_2 [9]. As another important material in microfabrication, SU-8 (negative photoresist) was initially used mainly as the material to prepare masters in PDMS device fabrications [10]; however, it has become increasingly popular as the material of choice to directly fabricate microfluidic devices in recent years [11]. For example, SU-8 has been used in waveguide applications, especially for optofluidic integrated devices [12]. It has been reported that SU-8 can be used as not only the wall material in microfluidic channels but also optical lenses integrated within the microchannel system for optical detection [13]. Hybrid microfluidic devices made of both SU-8 and PDMS have also attracted a significant amount of attention because they allow easy fabrication process to prepare complex microchannel features [2, 14, 15]. However, bonding between the SU-8 and PDMS surfaces has proven to be much more difficult [16, 17]. Moreover, SU-8/PDMS hybrid microfluidic system is susceptible to channel surface fouling, especially when it is used to handle samples that contain blood, proteins or cells [18]. Therefore, developing a facile method to prepare a SU-8/PDMS heterogeneous microfluidic system

with an excellent surface non-fouling performance is important to explore its full potentials in detection and diagnostic applications.

In our previous study, we developed a method to bond SU-8 and PDMS surfaces to prepare a hybrid microfluidic device and simultaneously modified both SU-8 and PDMS surfaces of the hybrid device using poly(ethylene glycol) (PEG) to enhance its surface non-fouling properties [19]. In the current study, poly(amidoamine) (PAMAM) dendrimers were used to modify the surfaces of the hybrid microfluidic channels, aiming to further improve the non-fouling performance of the SU-8/PDMS hybrid device. PAMAM dendrimers are mono-dispersed macromolecules with highly branched three-dimensional structures, which have been shown to not only cover larger surface area than linear molecules once grafted, but also provide numerous functional groups on the surface to be further functionalized [20-22]. We showed that the hybrid microchannel could be successfully fabricated and that the new hybrid PAMAM modified microchannel could reduce as much as 99.7% nonspecific hydrophobic microbeads adsorption, a significant performance increase from a reduction of 95.8% as we reported with the PEG modified hybrid microfluidic channels. This is a significantly improved non-fouling performance that has not been reported in the literature. In addition, we also demonstrated that the microfluidic system reduced *Escherichia coli* O157:H7 bacterial cell adsorption by as much as 95%, a much improved non-fouling performance comparable with other reports in the literature. In microfluidic systems, PAMAM dendrimers have been used to either provide high surface area because of their molecular structures [23] or improve the sensitivity of microfluidic biosensors due to their multi-functional end groups that allow more loading capacity of biomarkers [24].

However to our best knowledge, there is no report focusing on the non-fouling property of PAMAM dendrimers in microfluidic systems and articles reporting ways to employ PAMAM for SU-8/PDMS hybrid device fabrications, which is of significant importance for microfluidic systems for applications in diagnostic and biosensing [25].

3.3 Experimental

3.3.1 Materials

PAMAM dendrimer solution (generation 7, ethylenediamine core) (PAMAM-NH₂, 536725) was obtained from Sigma-Aldrich (Oakville, ON). Furthermore, the PAMAM-NH₂ was functionalized with carboxyl groups (i.e. PAMAM-COOH) using a method described elsewhere [26, 27] (see Appendix A for more information). Fluorescent fluoresbrite yellow green (YG) microbeads (17154, diameter 1.063 μm) were purchased from Polysciences Inc. (Warrington, PA). SU-8 negative photoresist was obtained from MicroChem (Newton, MA). Sylgard 184 PDMS kit was obtained from Dow Corning (Midland, MI). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC, A10807) was purchased from Alfa Aesar (Ward Hill, MA). (3-aminopropyl)-trimethoxysilane (APTMS, 313251000), *N*-hydroxysuccinimide (NHS, 157270250) and rhodamine-NHS (46406) were purchased from Thermo Scientific Inc. (Rockford, IL). Ethylenediamine anhydrous (EDA, E479-500) and toluene (T324-4) were purchased from Fisher Scientific Inc. (Ottawa, ON). Furthermore, formalin-killed fluorescein isothiocyanate (FITC) labeled *E. coli* O157:H7 (10^9 cells/mL,

43889) is the product of ATCC. All other chemicals were obtained from Sigma-Aldrich and used as received unless indicated otherwise.

3.3.2 General approach

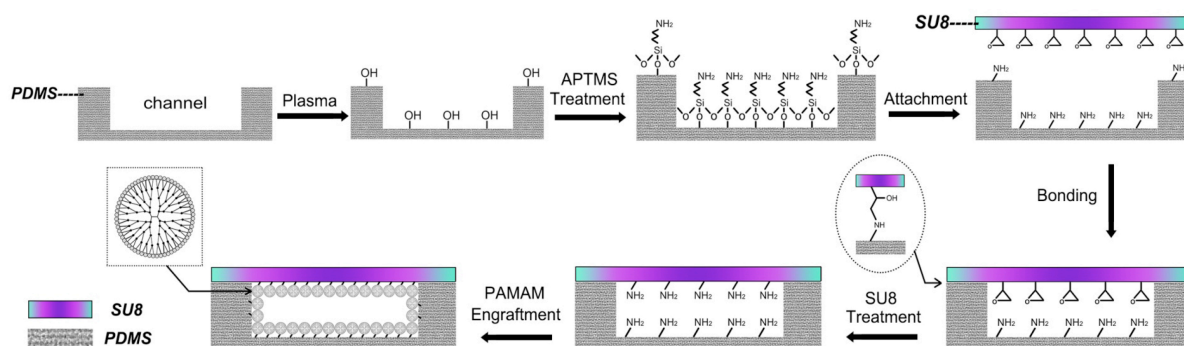


Figure 3-1. A schematic illustration outlining overall strategy to prepare PAMAM modified SU-8 and PDMS hybrid microfluidic channels.

As shown in Figure 3-1, our overall strategy is to first activate PDMS surface by oxygen plasma and subsequently amine functionalize the treated PDMS surface using APTMS through silanol condensation [28]. The resulting amine functionalized PDMS surface is then sealed with SU-8 through reactions between epoxy groups on the SU-8 surface and amino groups on the PDMS surface. The free SU-8 surface of the sealed microchannel is further amine-functionalized by EDA. Finally, PAMAM-COOH is simultaneously immobilized onto the free surfaces of both SU-8 and PDMS of the hybrid microfluidic channel via amino functional groups on both SU-8 and PDMS surfaces of the hybrid microchannels.

3.3.3 SU-8 and PDMS hybrid microfluidic channel preparation

To prepare PDMS surfaces, a standard soft lithography process was employed. Briefly, Sylgard 184 silicone elastomer base and its curing agent were well mixed at a ratio of 10:1 (w/w) and carefully poured onto a patterned SU-8 master. The mixture was degassed for 30 min and subsequently baked at 100 °C for 2 h, after which the molded PDMS was peeled off from the master. A PDMS flat surface without patterns was similarly prepared using a 4-inch bare silicon wafer instead of a patterned SU-8 master. Likewise, to prepare a plain SU-8 plate without patterns, the whole SU-8 surface was exposed to UV light without the use of a photomask.

3.3.4 Surface amination

To introduce amino groups to the PDMS surfaces, a silane coupling method was used with minor modifications [29, 30]. Briefly, PDMS surfaces were oxygen plasma treated with a plasma cleaner (SP100, Anatech Ltd, Battle Creek, MI) at 100 mTorr, 118 W for 10 s. Immediately after the plasma treatment, the activated PDMS surfaces were treated with APTMS solution for 20 s to allow PDMS surface amination by silanol condensation. Five APTMS concentrations (0.02, 0.05, 0.2, 0.5 and 1 wt.%) in toluene were evaluated in this study. Finally the PDMS surfaces were air dried for 30 min.

To aminate SU-8 surfaces, the SU-8 surfaces were treated with EDA ethanol solution for 1 h and then incubated at 70 °C for another 1 h. This allowed the epoxy groups on the SU-8 surfaces to react with the amino groups on one end of the bifunctional EDA molecules, resulting in amine functionalized SU-8 surfaces for further PAMAM engraftment.

3.3.5 Hybrid device fabrication

To prepare a functional SU-8/PDMS hybrid microfluidic device, the APTMS modified PDMS surface was first sealed with a SU-8 surface under 1.6×10^{-2} MPa pressure at 105 °C for 30 min. Subsequently, stainless steel tubes were inserted at terminals of the microchannels and secured by pressure sealing at 135 °C for another 30 min. The obtained SU-8/PDMS hybrid microchannel ($150 \mu\text{m} \times 20 \mu\text{m} \times 5 \text{ cm}$, $W \times D \times L$) was further treated with EDA solution for 1 h and then incubated at 70 °C for another 1 h to allow SU-8 surface amination.

To immobilize PAMAM onto both SU-8 and PDMS surfaces at the same time, both amine functionalized surfaces (i.e. SU-8 and PDMS) were incubated with PAMAM-COOH, 1.74 mM NHS and 1.04 mM EDC in 0.1 M 2-(4-morpholino)-ethane sulfonic acid (MES) solution (pH 6.0) at 35 °C for 2 h. Subsequently, the modified surfaces were washed using PBS (pH 7.4) at a flow rate of 0.05 mL/h for 10 min and finally stored PBS (pH 7.4) for further analysis. Three PAMAM-COOH concentrations (i.e. 1.5, 4.0 and 8.0 μM) were evaluated.

3.3.6 Surface characterizations

3.3.6.1 Surface amination characterization

To estimate the level of amination on both SU-8 and PDMS surfaces, the aminated surfaces were fluorescently labeled utilizing reactions between primary amines (on microchannel surfaces) and fluorescent rhodamine-NHS. In theory, the amount of amines on the surface is proportional to the amount of rhodamine-NHS immobilized on the surfaces of interest and the relative amount of immobilized rhodamine-NHS can be conveniently measured by the relative intensity of fluorescence emitted by the labeled surfaces. This method is an approximate approach for evaluating the relative amount of functional groups of interest on a surface. Briefly, in a typical experiment 10 mg/mL rhodamine-NHS in anhydrous dimethylformamide (DMF) was mixed with PBS (pH 7.4) at a proportion of 500 μ L PBS per μ L rhodamine-NHS DMF solution. The resulting labeling solution was used to react with the surfaces of interest at room temperature for 30 min, after which the surfaces were washed using PBS (pH 7.4) at a flow rate of 0.05 mL/h for 10 min and stored in PBS (pH 7.4) for future analysis. To analyze fluorescence intensity on the surfaces, fluorescence images were first captured by an inverted fluorescence microscope (Olympus IX81, Richmond Hill, ON) equipped with a high-resolution camera (QImaging, Surrey, BC) and the original color images were converted to gray-scale and analyzed by Image-Pro Plus 6.0 software (Media Cybernetics Inc., Rockville, MD). The average relative intensities of the gray-scale images were proportional to the amount of the conjugated rhodamine-NHS on the surface of interest, therefore indirectly reflecting the relative amount of amines on the surface [19, 31, 32]. The fluorescence intensities of the unmodified surfaces before amination were used as controls.

3.3.6.2 Water contact angle measurements

To investigate PAMAM surface modifications, water contact angles of surfaces of interest were characterized using a goniometer (AST Products Inc., Billerica, MA) as we previously reported [20]. Briefly $\sim 10\ \mu\text{L}$ DI water was used in each measurement; to minimize water evaporation, measurements were taken within 10 s after needle tip withdrawal from the water droplet. For each sample surface, three measurements (2 readings per measurement, total of 6 readings) were taken at random (and different) locations on a sample surface, and the averaged values were reported.

3.3.6.3 Characterization of PAMAM engraftment

To further study PAMAM engraftment, surfaces were characterized by an X-ray photoelectron spectroscopy (XPS) (PHI 5500, Physical Electronics, Chanhassen, MN). The take-off angles for all measurements were fixed at 45° . For all XPS analysis, 285.0 eV was used as a reference position for C-H(C) peak in this study. High resolution spectra C 1s and N 1s peaks were analyzed by using XPSPEAK software Version 4.1.

3.3.6.4 AFM surface morphology study

To characterize morphologies of the modified surfaces, a Veeco Di Multimode V atomic force microscope (AFM) (Santa Barbara, CA) was used. Surfaces of interest were analyzed in contact mode and then flattened by a NanoScope software (Veeco, Santa Barbara, CA). Data of roughness of all surfaces were also processed by the NanoScope software.

3.3.7 Hybrid microfluidic system non-fouling performance evaluation

To evaluate the effect of PAMAM modified device to prevent nonspecific adsorption, hydrophobic fluorescent poly (styrene) YG beads were initially used as model particles in this study (The microbeads were washed using a method described in Appendix A). Briefly, hydrophobic YG beads polybeads (1×10^8 particles/mL) were injected into a PAMAM-modified hybrid microchannel for 30 min at 0.05 mL/h using a syringe pump (Harvard Apparatus, Holliston, MA) [19]. This was followed by a washing step using PBS (pH 7.4) for 10 min at 0.05 mL/h. Finally, fluorescent microbeads adsorbed on the surfaces inside the microchannels were manually counted by Image J Version 1.46 (NIH, Bethesda, MD). In addition, FITC labeled *E. coli* O157:H7 cells were also used to further characterize the non-fouling performance of the hybrid microfluidic channels, under both dynamic and static flow conditions. For dynamic flow conditions, different concentrations of cell suspensions (i.e. 1×10^8 cells/mL, 1×10^6 cells/mL and 1×10^4 cells/mL) were separately pumped into the PAMAM modified hybrid microchannels at a volumetric flow rate of 0.05 mL/h for 20 h [19]. Subsequently the channels were carefully washed using PBS (pH 7.4) for 10 min at 0.05 mL/h. Finally, fluorescent *E. coli* O157:H7 cells adsorbed on the surfaces inside of the microchannels were manually counted as described above. Similarly for static flow

conditions, all three different concentrations of cell suspensions were injected into the PAMAM modified hybrid microchannels, and the cell suspensions were left inside of the channels for 10 min [19]. This was followed by the same washing methodology employed in the dynamic condition tests. Finally, fluorescent *E. coli* O157:H7 cells adsorbed on the surfaces inside of the microchannels were manually counted. In all tests, unmodified hybrid microchannels that underwent the same procedures were used as controls.

3.4 Results and discussion

3.4.1 Surface amination

Fluorescence labeling was used to evaluate the extent of surface aminations on both SU-8 and PDMS surfaces. While this is not an absolute method to quantify functional groups of interest on a surface, it has been well established as one of the most effective ways to study surface functional groups [31, 32]. Relative fluorescence intensities of aminated SU-8 and PDMS surfaces are shown in Figure 3-2. As shown in Figure 3-2(a), the relative fluorescence intensities showed a generally increasing trend with increasing APTMS concentrations, suggesting increasing aminations on oxygen plasma pretreated PDMS surfaces. However, it is worthwhile to note that higher levels of amination did not necessarily translate into better surface boning with SU-8 surface.

Table 3-1. Effects of APTMS concentration and oxygen plasma treatment on the bonding strength between PDMS and SU-8 surfaces.

Oxygen Plasma Time (s)	APTMS Conc. (wt.%)				
	0.02	0.05	0.2	0.5	1.0
10	⑤	⑤	①	①	①
15	④	⑤	①	①	①
20	②	③	①	①	①

- ① the surfaces easily fell apart.
- ② the surfaces detached at low tensile load.
- ③ the surfaces detached at moderate tensile load.
- ④ the surfaces detached at high tensile load.
- ⑤ the surfaces did not detach, PDMS had to be destroyed to peel off from SU-8 surface.

As it is evident in Table 1, only PDMS surfaces treated with oxygen plasma for 10 s or 15 s and subsequently modified with 0.05 wt.% APTMS strongly bonded with SU-8 surfaces; in these two cases the formed interface was so strong that the PDMS had to be destroyed before the SU-8/PDMS interface could be peeled apart. In contrast, higher aminations as a result of higher APTMS concentration treatments (i.e. 0.2 wt.%, 0.5 wt.% and 1.0 wt.%) achieved weaker SU-8 and PDMS bonding. Therefore it was concluded that PDMS surface treated with oxygen plasma for 10 s and subsequently modified with 0.05 wt.% APTMS

formed the strongest interface with SU-8. As a result, this experimental condition was used in the rest of this study unless indicated otherwise.

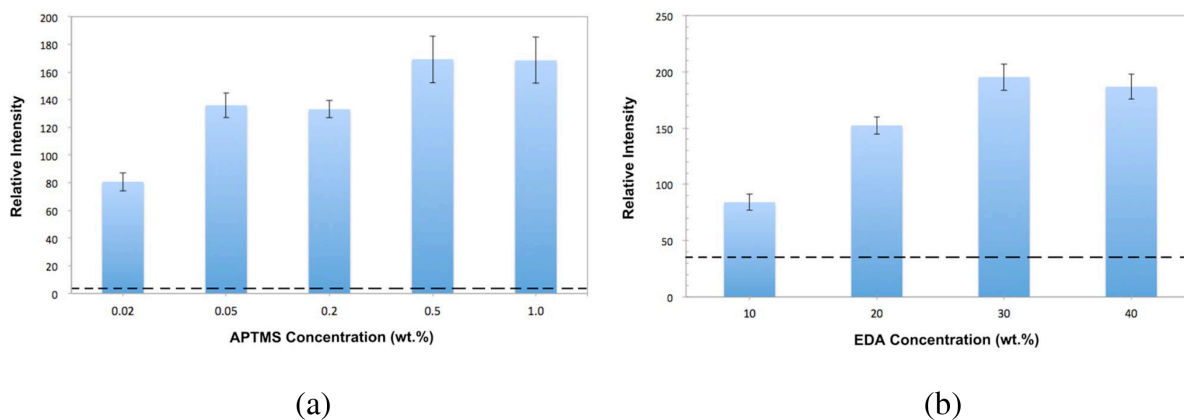


Figure 3-2. PDMS and SU-8 surface amination as determined by relative fluorescence intensity. (a) Effect of APTMS concentrations on PDMS surface amination. (b) Effect of EDA concentrations on SU-8 surface amination. Note: dotted line indicates relative fluorescence intensity of an unmodified PDMS or SU-8 surface (exposure time and other parameters are all the same); error bars indicate standard deviation (n=6).

To aminate SU-8 surfaces, EDA was used. The amino group on one end of a bifunctional EDA molecule reacts with an epoxy group on an SU-8 surface, leaving the other amino group for further PAMAM surface modifications. Figure 3-2(b) shows the effect of EDA concentration on SU-8 surface amination. It shows that initially aminations on SU-8 surfaces increased with increasing EDA concentrations till 30 wt.%, after which amination

leveled off as further increases in EDA concentration did not result in significant changes in SU-8 surface amination ($p=0.113$, one-way ANOVA). While 30 wt.% EDA solution treatment resulted in higher SU-8 surface amination as demonstrated by the highest relative fluorescence intensity amongst all EDA concentrations studied, it was also observed that microchannels were most often blocked if EDA concentrations were higher than 20 wt.%. Therefore, an EDA solution at 20 wt.% was used in the rest of this study unless indicated otherwise. It is noted that the background fluorescence (dotted line) is higher in the case of SU-8 surfaces in comparison with PDMS surfaces. This is likely because SU-8 surface is slightly auto-fluorescent at several wavelengths [33, 34].

3.4.2 Surface characterizations

To monitor the success of PAMAM modification on PDMS surfaces, water contact angle measurements were used. As shown in Figure S1 (see Appendix A), it is evident that the original PDMS surface was successfully amine functionalized and subsequently modified by PAMAM-COOH because water contact angles showed corresponding changes, as expected. In addition, a PAMAM-COOH concentration of 4.0 μM was determined to be the optimal PAMAM-COOH concentration to use in surface modifications, as further increase in the PAMAM-COOH did not result in any significant changes in water contact angle of the resulting surface ($p=0.192$, one-way ANOVA).

To further characterize PAMAM-grafted both SU-8 and PDMS surfaces, XPS and AFM were also used. Two PDMS surface samples (unmodified and PAMAM modified PDMS surfaces)

and two SU-8 surface samples (unmodified and PAMAM modified surfaces) were studied by XPS.

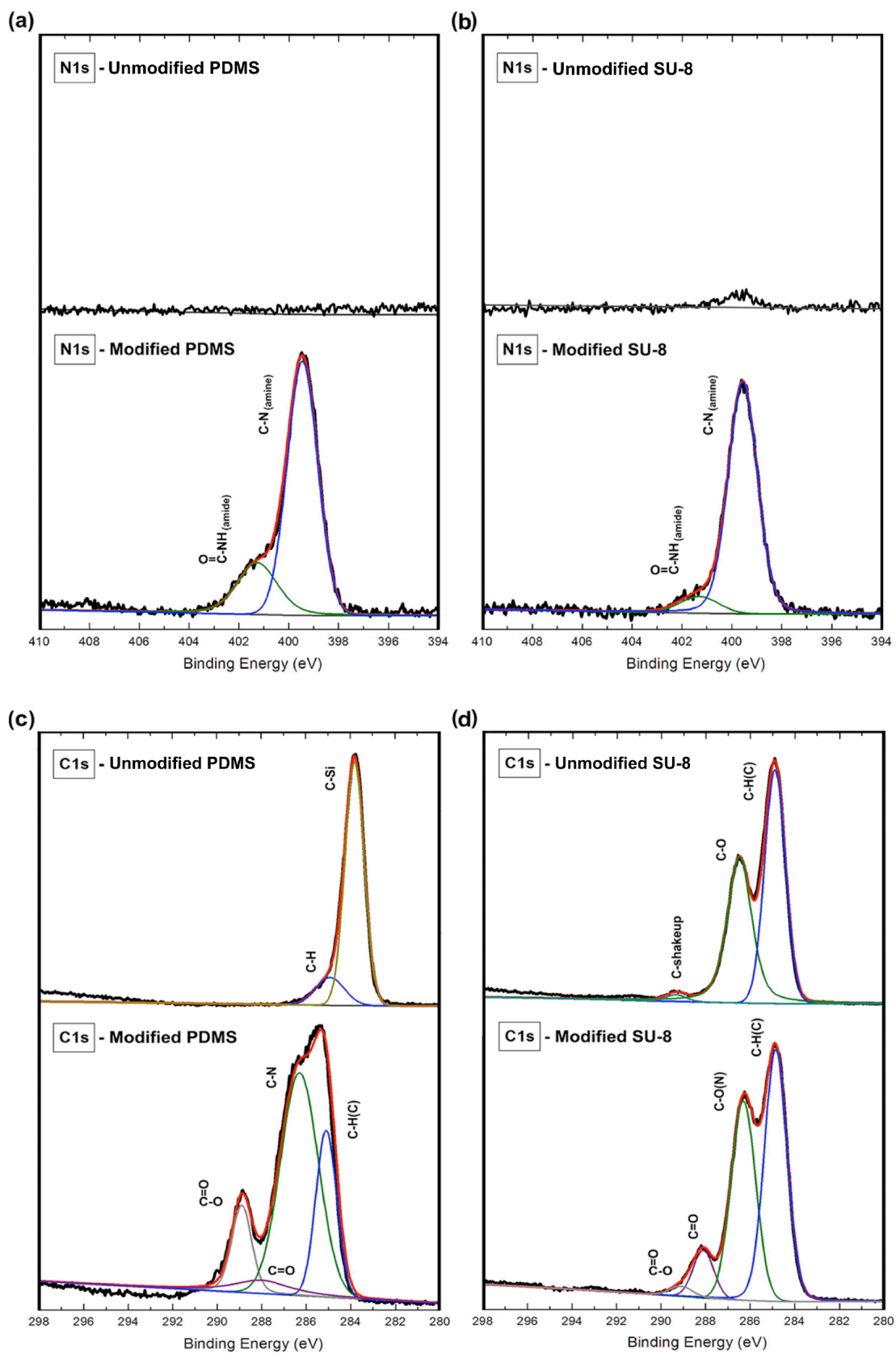
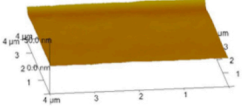
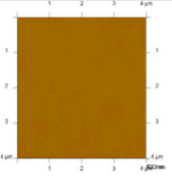
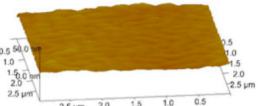
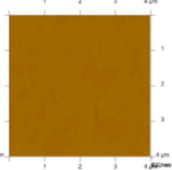
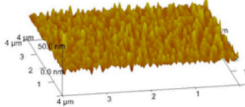
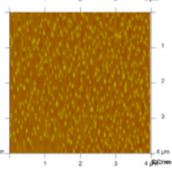
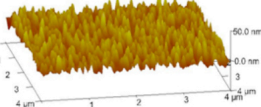
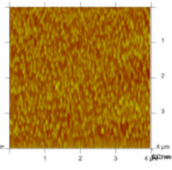
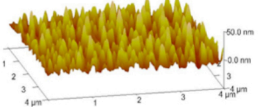
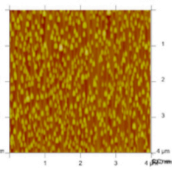


Figure 3-3. High resolution XPS spectra of (a) N 1s of unmodified and modified PDMS surfaces, (b) N 1s of unmodified and modified SU-8 surfaces, (c) C 1s of unmodified and modified PDMS surfaces, and (d) C 1s of unmodified and modified SU-8 surfaces.

Typical N 1s and C 1s XPS peaks of four surfaces of interest (i.e. unmodified PDMS and SU-8 surfaces, and PAMAM modified PDMS and SU-8 surfaces) are shown in Figure 3-3. The peaks in Figure 3-3 were deconvoluted according to literature reports [35]. As shown in Figures 3-3(a) and 3(b), it is evident that after PAMAM surface modification, the N 1s peaks showed dramatic differences before and after surface modification. The atomic composition of nitrogen on the PDMS and SU-8 surfaces increased to 8.9% and 7.1% from their original values of 0.0% and 0.0%, respectively. This confirms the introduction of nitrogen on the PAMAM-coated SU-8 and PDMS surfaces. In addition, the N 1s peaks of both SU-8 and PDMS modified surfaces were further decomposed into two components. The most prominent constituent peak at 401.2 eV can be attributed to amide bond -CONH- [36], which is consistent with the amide bonds formed between both PDMS and SU-8 surface amines (i.e. after amination) and the amide bonds intrinsic in the PAMAM. This suggests that both PDMS and SU-8 surfaces have been successfully modified with PAMAM dendrimer. The other smaller peak at approximately 399.7 eV can be attributed to amines C-N(H₂) [36], likely because of the combined contribution due to the amine from the original PAMAM-NH₂ and the unreacted amines on both SU-8 and PDMS surfaces. Similarly, XPS spectra of PAMAM-grafted samples also showed significant changes in C 1s peaks before and after surface modification. In Figure 3-3(c) the constituent of C-Si at 283.7 eV

was predominant on the unmodified PDMS surface whereas the C-Si peak almost disappeared after surface modification, as expected [37]. Furthermore, the C 1s peak of the PAMAM modified PDMS surface could be decomposed into four constituents: first constituent at approximately 285.0 eV for C-H(C) [38], second constituent at 286.4 eV for bond C-N, the third constituent at 288 eV for C=O [35] and the last constituent at about 289.1 eV for (C=O)-O [39]. During surface modification, the component of C-N increased dramatically and became dominant on the PAMAM-coated PDMS surface (56.8% on PAMAM-grafted PDMS surface) as demonstrated in Figure 3-3(c). The increase of the atomic composition of N, the decline of the atomic composition of element Si and the appearance of C=O peak at 288 eV and (C=O)-O peak at 289.1 eV are strong evidence of successful PAMAM surface modification on PDMS. Likewise, for unmodified SU-8 surface, the high-resolution C 1s spectrum (in Figure 3-3(d)) can be decomposed into three constituents: first at approximately 285.0 eV due to C-H(C), second near 286.4 eV due to C-O and energy loss of the shakeup features at 289.4 eV [39, 40]. In comparison, the C 1s peak of PAMAM grafted SU-8 surface was decomposed into four constituents: (1) C-H(C) at 285.0 eV, (2) C-O(N) at about 286.4 eV, (3) C=O at about 288 eV and (4) last constituent at 289.1 eV due to (C=O)-O. It should be noted that two additional C 1s peaks (at 288 eV and 289.1 eV) appeared after the PAMAM modification – most likely due to 1) the formation of new amide bonds generated between EDA and PAMAM-COOH and 2) the amide bonds intrinsic to PAMAM – both of which strongly suggesting that the PAMAM was successfully grafted to the SU-8 surface.

(a)

Groups		AFM 3D Image	Vertical View	Roughness
Unmodified PDMS				0.28 ± 0.06
PDMS with APTMS				1.16 ± 0.09
PAMAM-COOH Conc. (μM)	1.5			3.72 ± 0.11
	4.0			4.49 ± 0.30
	8.0			6.59 ± 0.37

(b)

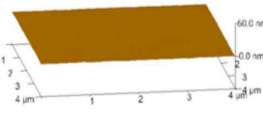
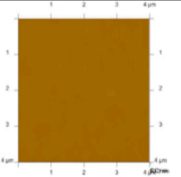
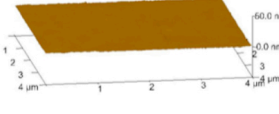
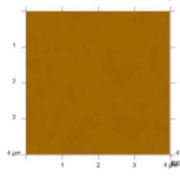
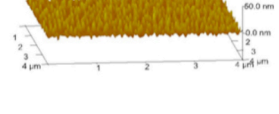
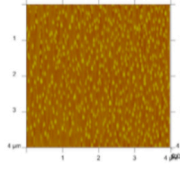
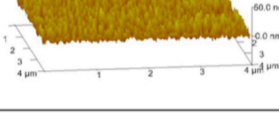
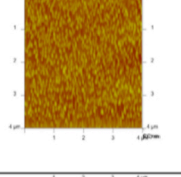
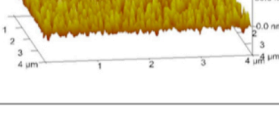
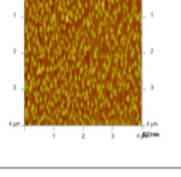
Groups		AFM 3D Image	Vertical View	Roughness
Unmodified SU-8				0.26 ± 0.02
SU-8 with EDA				0.31 ± 0.02
PAMAM-COOH Conc. (μ M)	1.5			3.03 ± 0.09
	4.0			4.70 ± 0.05
	8.0			6.31 ± 0.32

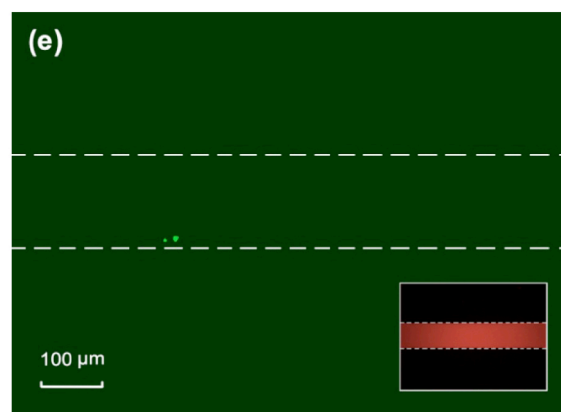
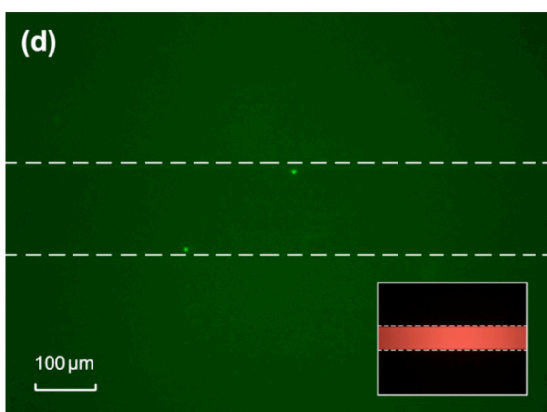
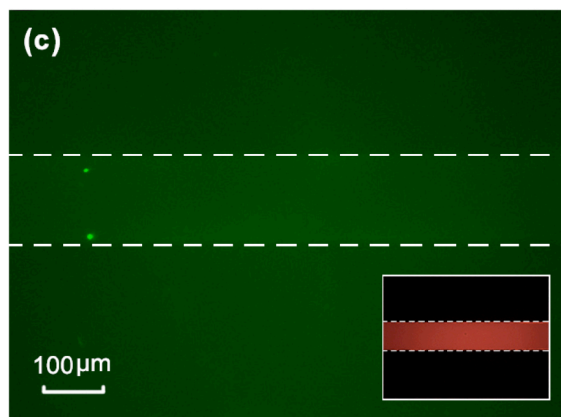
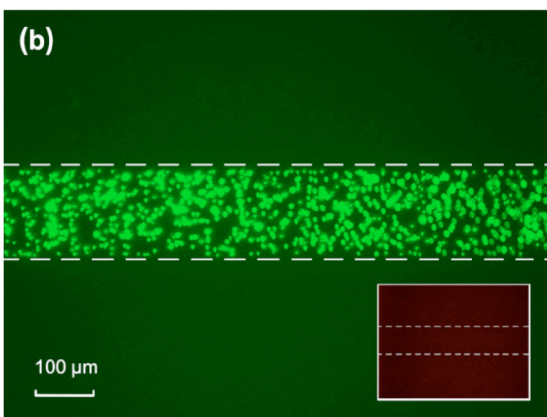
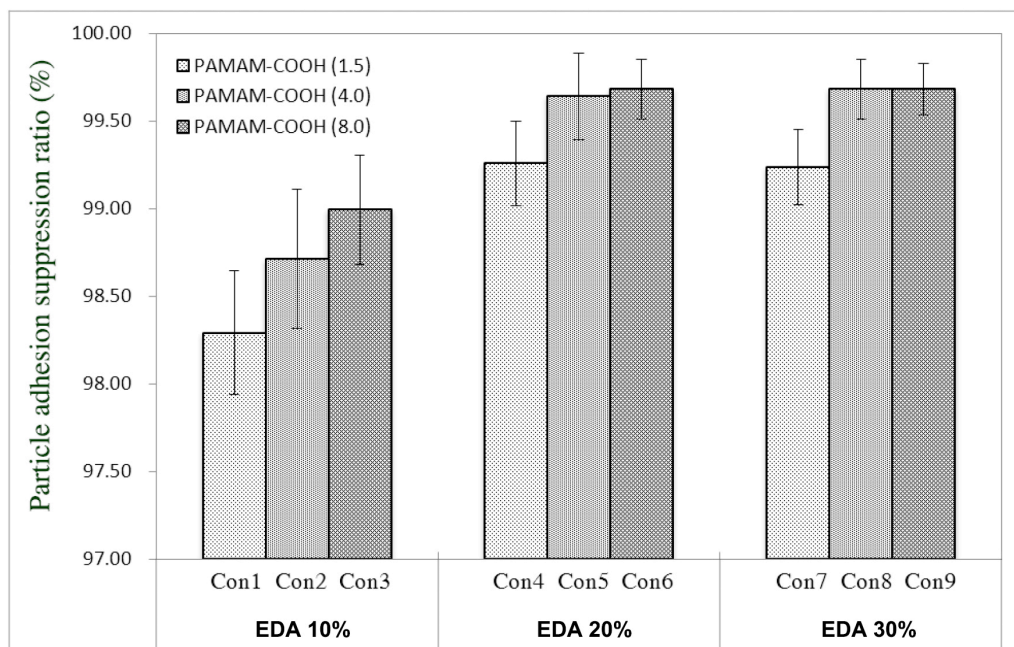
Figure 3-4. AFM images of (a) SU-8 surfaces in each of the surface modification steps and (b) PDMS surfaces in each of the surface modification steps. The roughness of each surface was calculated to indicate relative surface roughness. Note that the relative higher edge shown in unmodified PDMS was because of the distortion of PDMS surface during AFM scanning. Standard deviation of the measurements was shown as error bars (n=4).

The morphologies of PDMS and SU-8 surfaces in each step of modification were investigated by AFM in contact mode. As shown in Figure 3-4, both SU-8 and PDMS surface topographies changed significantly immediately after PAMAM surface modifications while treatments of SU-8 and PDMS surfaces with respective EDA and APTMS did not significantly change surface morphologies. This surface morphology change is likely due to the 3D molecular packing of PAMAM dendrimers on SU-8 and PDMS surfaces [41], and the significant surface roughness changes also strongly suggests that PAMAM dendrimers have been successfully conjugated onto both SU-8 and PDMS surfaces. Moreover, it is interesting to note that the roughness of PAMAM modified surfaces increased with increasing PAMAM concentration used to modify the surfaces. This is likely due to increased PAMAM dendrimer aggregation on the resulting surfaces [42, 43]. In addition, a closer analysis of the AFM images reveals that almost all SU-8 surfaces were more uniform and better organized than the PDMS surfaces. This agrees well with our previous observation in another study [19], and we believe it is likely because of the relatively hydrophilic nature of SU-8 surface than PDMS surface, as the reported water contact angle of unmodified SU-8 is about 80 ° while PDMS is around 110 ° [44, 45].

3.4.3 Non-fouling performance of hybrid microchannels

To test the non-fouling performance of the modified hybrid microchannels, yellow green fluorescent polystyrene microbeads were initially used as model particles in non-specific particle adhesion studies.

(a)



(f)

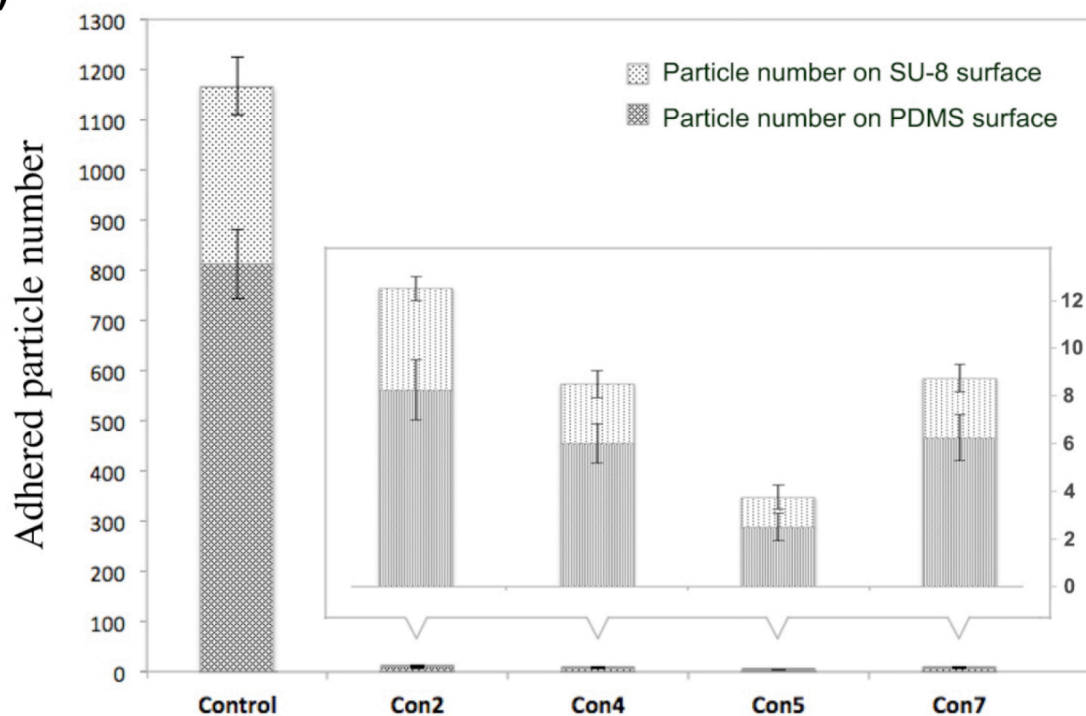


Figure 3-5. Non-fouling performance of hybrid microchannels in particle adhesion studies using fluorescent microbeads. (a) Particle adhesion suppression performance of different microchannels using various surface modification conditions; error bars indicate standard deviations (n=4). (b) Fluorescence images of non-fouling performance of hybrid microchannels without modification, (c) microchannel treated with Con 5, (d) microchannel treated with Con 6, (e) microchannel treated with Con 8. Note fluorescent microbeads are shown in green and insets show fluorescence image of hybrid microchannels after modifications with PAMAM-COOH labeled with rhodamine as shown in red. (f) Particle adsorption on SU-8 and PDMS surfaces in hybrid microchannels; error bars indicate standard deviations (n=4).

As shown in Figures 3-5, it is evident that all investigated PAMAM modified microchannels showed significantly improved non-fouling properties in comparison with unmodified microchannels. It is worthwhile mentioning that a particle suppression of more than 99.5% was routinely achieved by employing surface modification Conditions 5, 6, 8 and 9. The best particle suppression of 99.7% was achieved with Conditions 6, 8 and 9 at 99.7%. This is a significant performance improvement over our previous effort to use linear PEG to improve the non-fouling properties of the hybrid microchannels that achieved a particle suppression value of 95.8% [19]. In addition, Figures 3-5(b) to (e) show fluorescence images of some of the tests whose results are summarized in Figure 3-5(a). Fluorescently tagged PAMAM was used to functionalize the hybrid microchannels (shown in insets) in these tests (data from microbead adhesion studies are shown in Table S1 in Appendix A). A closer look at the data shows that SU-8 surfaces appear to adsorb fewer particles than PDMS surfaces under the same conditions (see Figure 3-5(f)). This is likely due to better PAMAM surface organizations on SU-8 than on PMDS surfaces as shown in AFM results (see Figure 3-4).

(a)

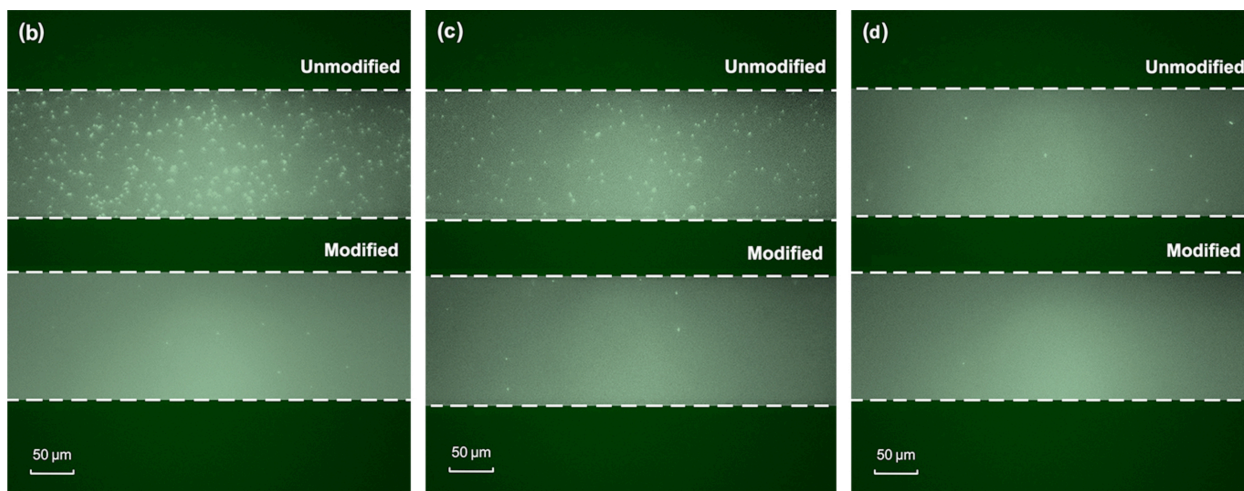
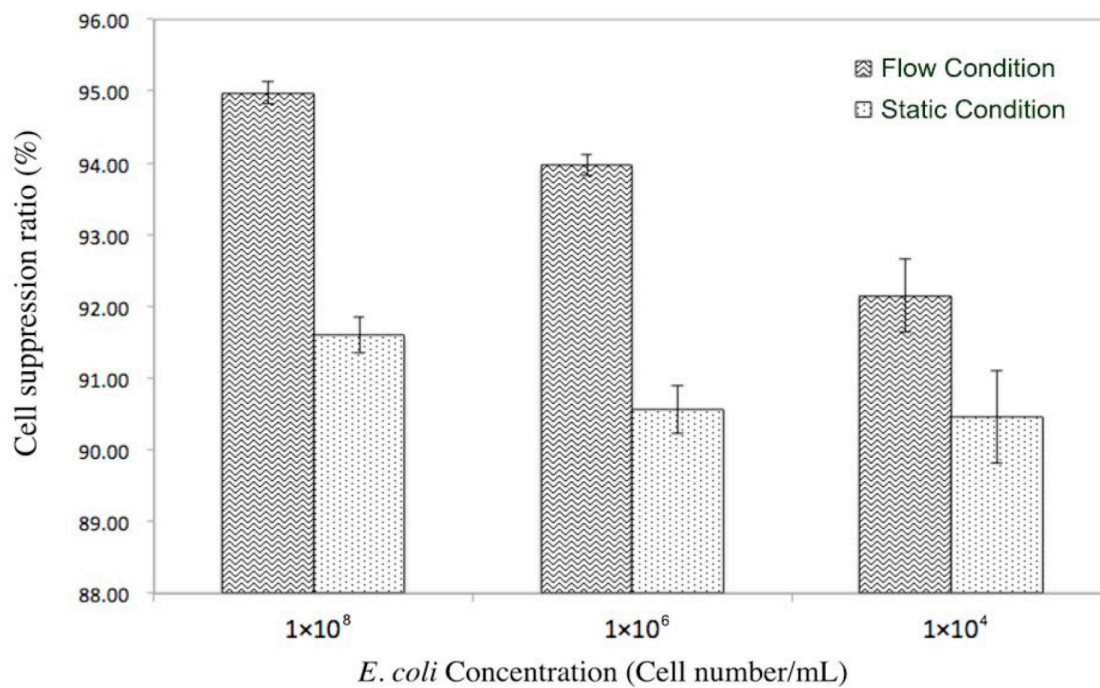


Figure 3-6. (a) Non-fouling performance of hybrid microchannels in *E. coli* O157:H7 adhesion studies; error bars indicate standard deviations (n=4). Fluorescence images of non-fouling performance of both unmodified and PAMAM modified hybrid microchannels

in flow condition studies with different *E. coli* concentrations of (b) 1×10^8 cells/mL, (c) 1×10^6 cells/mL, (d) 1×10^4 cells/mL. Fluorescent *E. coli* cells are shown in green.

To further demonstrate the non-fouling performance of the microchannels, *E. coli* 0157:H7 cell suspensions were injected into the microchannels under both flow conditions and static conditions. The flow rate used in *E. coli* studies was 0.05 mL/h. As shown in Figure 3-6 (a), cell adhesion suppression was consistently higher than 90% for all cell concentrations studied and the highest adhesion suppression was around 95%, a result that is consistent with other reported efforts to reduce surface fouling by *E. coli* [46]. As expected, for a given cell concentration, cell adhesion suppression is higher in flow conditions than in static conditions, most likely due to the effect of shear stress [47]. In addition, Figures 3-6 (b) to (d) show the performances of unmodified and PAMAM modified microchannels in flow conditions at different *E. coli* concentrations. It is interesting to note that the cell suppression ratio increases with increasing cell concentrations in suspension, and this trend is true for both flow and static conditions. This is likely because with increasing cell concentrations in suspension, the number of nonspecifically adherent cells on unmodified microchannel surfaces (N_{unmod}) increased much more dramatically than that on modified microchannel surfaces (N_{mod}), thus decreasing the ratio of adherent cell number on modified surfaces vs. unmodified surfaces (N_{mod} / N_{unmod}), and subsequently increased the suppression ratio ($1 - (N_{mod} / N_{unmod})$). This is further supported by unspecific cell adhesion data as shown in Table S2 in Appendix A.

3.5 Conclusions

In this study, we show a facile strategy to bond SU-8 and PDMS surfaces to prepare a hybrid microchannel and simultaneously modify the surface of resulting microchannel to enhance its surface non-fouling properties for potential applications in biosensor and detections. We show that by using PAMAM dendrimer to modify the hybrid microchannel surfaces, the microfluidic system exhibits a greatly improved non-fouling performance of up to 99.7% hydrophobic microbead particle suppression. In addition, the same PAMAM modified microfluidic system also shows 95% non-fouling performance for *E. coli* bacterial cell adsorption suppression. As a result, we believe the ease of making a hybrid microfluidic system combined with the simple chemistry involved to simultaneously surface modify both PDMS and SU-8 of the hybrid device makes this strategy an attractive approach to prepare lab-on-chip microfluidic systems for biosensing and detection applications.

3.6 References

- [1] S. Bhattacharya, A. Datta, J.M. Berg, S. Gangopadhyay, Studies on surface wettability of poly (dimethyl) siloxane (PDMS) and glass under oxygen-plasma treatment and correlation with bond strength, *Microelectromechanical Systems, Journal of*, 14 (2005) 590-597.
- [2] C.-F. Wan, C.-S. Chen, K.-C. Hwang, Y.-H. Lai, J.-Y. Luo, P.-C. Liu, L.-S. Fan, Development and automation of microelectromechanical systems-based biochip platform for protein assay, *Sensors and Actuators B: Chemical*, 193 (2014) 53-61.
- [3] W. Liu, X. Zhou, D. Xing, Rapid and reliable microRNA detection by stacking hybridization on electrochemiluminescent chip system, *Biosensors and Bioelectronics*, 58 (2014) 388-394.
- [4] J. Horak, C. Dincer, H. Bakirci, G. Urban, Sensitive, rapid and quantitative detection of substance P in serum samples using an integrated microfluidic immunochip, *Biosensors and Bioelectronics*, 58 (2014) 186-192.
- [5] Q. Tu, J.-C. Wang, Y. Zhang, R. Liu, W. Liu, L. Ren, S. Shen, J. Xu, L. Zhao, J. Wang, Surface modification of poly (dimethylsiloxane) and its applications in microfluidics-based biological analysis, (2012).
- [6] P.N. Nge, C.I. Rogers, A.T. Woolley, Advances in microfluidic materials, functions, integration, and applications, *Chemical reviews*, 113 (2013) 2550-2583.
- [7] S. Oh, Thick single-layer positive photoresist mold and poly (dimethylsiloxane)(PDMS) dry etching for the fabrication of a glass-PDMS-glass microfluidic device, *Journal of Micromechanics and Microengineering*, 18 (2008) 115025.
- [8] K.-P. Zimmer, A. Braun, M. Kostrzewa, Shrinking the apparatus size for DNA analysis, *Smart Materials and MEMS, International Society for Optics and Photonics*, 2001, pp. 58-65.
- [9] C. Séguin, J.M. McLachlan, P.R. Norton, F. Lagugné-Labarhet, Surface modification of poly (dimethylsiloxane) for microfluidic assay applications, *Applied Surface Science*, 256 (2010) 2524-2531.
- [10] P.-Y. Yeh, N.A. Rossi, J.N. Kizhakkedathu, M. Chiao, A silicone-based microfluidic chip grafted with carboxyl functionalized hyperbranched polyglycerols for selective protein capture, *Microfluidics and Nanofluidics*, 9 (2010) 199-209.
- [11] E. Zeller, A. Mitchell, Sealed air-core planar waveguide arrays in SU8 epoxy, *Smart Materials, Nano-and Micro-Smart Systems, International Society for Optics and Photonics*, 2008, pp. 72690E-72690E-72699.
- [12] H. Kotari, M. Motosuke, S. Honami, Particle sorting by optical radiation pressure with low energy density, *La Houille Blanche*, (2013) 72-78.
- [13] B.R. Watts, Z. Zhang, C.Q. Xu, X. Cao, M. Lin, Scattering detection using a photonic - microfluidic integrated device with on - chip collection capabilities, *Electrophoresis*, 35 (2014) 271-281.
- [14] M.S. Cheri, H. Latifi, J. Sadeghi, M.S. Moghaddam, H. Shahraki, H. Hajghassem, Real-time measurement of flow rate in microfluidic devices using a cantilever-based optofluidic sensor, *Analyst*, 139 (2014) 431-438.
- [15] F. Perdignes, J.M. García, J.M. Quero, Fabrication process for integration of SU-8 check valves on printed circuit board-based microfluidic platforms, *IET Micro & Nano Letters*, 9 (2014) 37-40.

- [16] Z. Zhang, P. Zhao, G. Xiao, B.R. Watts, C. Xu, Sealing SU-8 microfluidic channels using PDMS, *Biomicrofluidics*, 5 (2011) 046503.
- [17] C.-W. Tsao, D.L. DeVoe, Bonding of thermoplastic polymer microfluidics, *Microfluidics and Nanofluidics*, 6 (2009) 1-16.
- [18] Q. Tu, J.-C. Wang, R. Liu, J. He, Y. Zhang, S. Shen, J. Xu, J. Liu, M.-S. Yuan, J. Wang, Antifouling properties of poly (dimethylsiloxane) surfaces modified with quaternized poly (dimethylaminoethyl methacrylate), *Colloids and Surfaces B: Biointerfaces*, 102 (2013) 361-370.
- [19] P.Y. Yeh, Z. Zhang, M. Lin, X. Cao, Nonfouling hydrophilic poly (ethylene glycol) engraftment strategy for PDMS/SU-8 heterogeneous microfluidic devices, *Langmuir*, 28 (2012) 16227-16236.
- [20] N. Cheng, X. Cao, Photoactive SAM surface for control of cell attachment, *Journal of colloid and interface science*, 348 (2010) 71-79.
- [21] P.-Y.J. Yeh, R.K. Kainthan, Y. Zou, M. Chiao, J.N. Kizhakkedathu, Self-assembled monothiol-terminated hyperbranched polyglycerols on a gold surface: a comparative study on the structure, morphology, and protein adsorption characteristics with linear poly (ethylene glycol) s, *Langmuir*, 24 (2008) 4907-4916.
- [22] V. Katur, M. Eichler, E. Deigle, C. Stage, P. Karageorgiev, J. Geis-Gerstorfer, G. Schmalz, S. Ruhl, F. Rupp, R. Müller, Surface-immobilized PAMAM-dendrimers modified with cationic or anionic terminal functions: Physicochemical surface properties and conformational changes after application of liquid interface stress, *Journal of colloid and interface science*, 366 (2012) 179-190.
- [23] S. Prakash, T.M. Long, J. Wan, J.S. Moore, M.A. Shannon, Electroosmotic Flow in “Click” Surface Modified Microfluidic Channels, *ASME 4th International Conference on Nanochannels, Microchannels, and Minichannels*, American Society of Mechanical Engineers, 2006, pp. 249-255.
- [24] W. Xu, Y. Wang, S. Liu, J. Yu, H. Wang, J. Huang, A novel sandwich-type electrochemical aptasensor for sensitive detection of kanamycin based on GR-PANI and PAMAM-Au nanocomposites, *New Journal of Chemistry*, 38 (2014) 4931-4937.
- [25] W.-I. Wu, K.N. Sask, J.L. Brash, P.R. Selvaganapathy, Polyurethane-based microfluidic devices for blood contacting applications, *Lab on a Chip*, 12 (2012) 960-970.
- [26] X. Shi, A.K. Patri, W. Lesniak, M.T. Islam, C. Zhang, J.R. Baker, L.P. Balogh, Analysis of poly (amidoamine) - succinamic acid dendrimers by slab - gel electrophoresis and capillary zone electrophoresis, *Electrophoresis*, 26 (2005) 2960-2967.
- [27] S. Uppuluri, D.R. Swanson, L.T. Piehler, J. Li, G.L. Hagnauer, D.A. Tomalia, Core-shell tecto (dendrimers): I. Synthesis and characterization of saturated shell models, *Advanced Materials*, 12 (2000) 796-800.
- [28] A.D. Stroock, G.M. Whitesides, Components for integrated poly (dimethylsiloxane) microfluidic systems, *Electrophoresis*, 23 (2002) 3461-3473.
- [29] E. Ouellet, C.W.T. Yang, T. Lin, L.L. Yang, E.T. Lagally, Novel carboxyl-amine bonding methods for poly (dimethylsiloxane)-based devices, *Langmuir*, 26 (2010) 11609-11614.
- [30] Z. Zhang, P. Zhao, G. Xiao, The fabrication of polymer microfluidic devices using a solid-to-solid interfacial polyaddition, *Polymer*, 50 (2009) 5358-5361.
- [31] R.K. Kainthan, Y. Zou, M. Chiao, J.N. Kizhakkedathu, Self-Assembled Monothiol-Terminated Hyperbranched Polyglycerols on a Gold Surface: A Comparative Study on the Structure, Morphology, and Protein Adsorption Characteristics with Linear Poly(ethylene glycol)s, *Langmuir*, 24 (2008) 4907-4916.

- [32] P.E. Scopelliti, A. Borgonovo, M. Indrieri, L. Giorgetti, G. Bongiorno, R. Carbone, A. Podesta, P. Milani, The effect of surface nanometre-scale morphology on protein adsorption, *PLoS One*, 5 (2010) e11862.
- [33] G. Blagoi, S. Keller, A. Johansson, A. Boisen, M. Dufva, Functionalization of SU-8 photoresist surfaces with IgG proteins, *Applied Surface Science*, 255 (2008) 2896-2902.
- [34] R. Marie, S. Schmid, A. Johansson, L. Ejlsing, M. Nordström, D. Häfliger, C.B.V. Christensen, A. Boisen, M. Dufva, Immobilisation of DNA to polymerised SU-8 photoresist, *Biosensors and Bioelectronics*, 21 (2006) 1327-1332.
- [35] E. Briand, V. Humblot, J. Landoulsi, S. Petronis, C.-M. Pradier, B. Kasemo, S. Svedhem, Chemical Modifications of Au/SiO₂ Template Substrates for Patterned Biofunctional Surfaces, *Langmuir*, 27 (2010) 678-685.
- [36] Z. Xu, T. Li, Z. Zhong, D.-s. Zha, S. Wu, F. Liu, W. Xiao, X. Jiang, X. Zhang, J. Chen, Amide-linkage formed between ammonia plasma treated poly(D,L-lactide acid) scaffolds and bio-peptides: Enhancement of cell adhesion and osteogenic differentiation in vitro, *Biopolymers*, 95 (2011) 682-694.
- [37] X. Zhao, Y. Su, Y. Li, R. Zhang, J. Zhao, Z. Jiang, Engineering amphiphilic membrane surfaces based on PEO and PDMS segments for improved antifouling performances, *Journal of Membrane Science*, 450 (2014) 111-123.
- [38] B.V. Crist, Handbook of monochromatic XPS spectra, The elements of native Oxides, Handbook of Monochromatic XPS Spectra, The Elements of Native Oxides, by B. Vincent Crist, pp. 548. ISBN 0-471-49265-5. Wiley-VCH, October 2000., 1 (2000).
- [39] F. Walther, P. Davydovskaya, S. Zürcher, M. Kaiser, H. Herberg, A.M. Gigler, R.W. Stark, Stability of the hydrophilic behavior of oxygen plasma activated SU-8, *Journal of Micromechanics and Microengineering*, 17 (2007) 524.
- [40] S. Prabakaran, S. Jayaraman, D. Minh, S. Sinha, The Role of Functional End Groups of Perfluoropolyether (Z-dol and Z-03) Lubricants in Augmenting the Tribology of SU-8 Composites, *Tribol Lett*, 56 (2014) 423-434.
- [41] G. Li, L. Shen, Y. Luo, S. Zhang, The effect of silver-PAMAM dendrimer nanocomposites on the performance of PVDF membranes, *Desalination*, 338 (2014) 115-120.
- [42] M.J. Jasmine, E. Prasad, Fractal growth of PAMAM dendrimer aggregates and its impact on the intrinsic emission properties, *The Journal of Physical Chemistry B*, 114 (2010) 7735-7742.
- [43] H.A. Essawy, H.A. Mohamed, Poly (amidoamine) dendritic structures, bearing different end groups, as adhesion promoters for urea-formaldehyde wood adhesive system, *Journal of Applied Polymer Science*, 119 (2011) 760-767.
- [44] A. Deepu, V.V.R. Sai, S. Mukherji, Simple surface modification techniques for immobilization of biomolecules on SU-8, *Journal of Materials Science: Materials in Medicine*, 20 (2009) 25-28.
- [45] R.A. Lawton, C.R. Price, A.F. Runge, W.J. Doherty Iii, S.S. Saavedra, Air plasma treatment of submicron thick PDMS polymer films: effect of oxidation time and storage conditions, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 253 (2005) 213-215.
- [46] B.-Y. Yu, J. Zheng, Y. Chang, M.-C. Sin, C.-H. Chang, A. Higuchi, Y.-M. Sun, Surface zwitterionization of titanium for a general bio-inert control of plasma proteins, blood cells, tissue cells, and bacteria, *Langmuir*, 30 (2014) 7502-7512.
- [47] G.J. Mahler, C.M. Frendl, Q. Cao, J.T. Butcher, Effects of shear stress pattern and magnitude on mesenchymal transformation and invasion of aortic valve endothelial cells, *Biotechnology and Bioengineering*, (2014).

**Chapter 4 Developing a non-fouling hybrid microfluidic
device for applications in circulating tumour cell
detections**

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4.1 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 CCRF-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.

4.2 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 (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 nanoparticles 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.

4.3 Experimental

4.3.1 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-NH₂) 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.

4.3.2 General approach

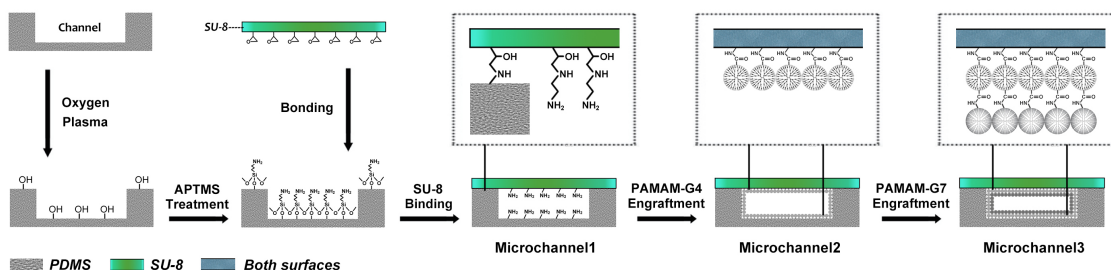


Figure 4-1. Schematic illustration outlining the overall strategy to prepare microfluidic channels of interest. Microchannel 1: surface functionalized by APTMS with amino groups; Microchannel 2: surface further functionalized by PAMAM-G4 dendrimers (carboxyl functionalized); Microchannel 3: surface finally functionalized by PAMAM-G7 dendrimers (amino functionalized).

As shown in Figure 4-1, oxygen plasma is used to activate PDMS surface, and subsequently APTMS is used to amine 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 amine 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-NH₂) is further immobilized onto both of the PAMAM-G4 modified PDMS and SU-8 surfaces in the microchannel to result in Microchannel 3.

4.3.3 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 inches) 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.

4.3.4 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 molecule, allowing one amino functional group to react with the epoxy group on the SU-8 surface while leaving the other amino group available to enable further PAMAM-G4 immobilization on the surface.

4.3.5 Hybrid device fabrication

In order to prepare an amino functionalized PDMS/SU-8 hybrid device, the aminated PDMS surface was first combined with an SU-8 surface (1.6×10^{-2} MPa pressure) at 110° C for 30 min. Subsequently stainless steel tubes were plugged into the ends of the microfluidic channel and baked at 130° C for 30 min to obtain a PDMS/SU-8 microchannel ($150 \mu\text{m} \times 50 \mu\text{m}$, $W \times D$). This was followed by introducing an EDA solution (20 wt.% in anhydrous alcohol) into the microchannel at a flow rate of 0.05 mL/h for 1 h. Finally the device was baked at 70° C for another hour to aminate the SU-8 surface.

In order to immobilize PAMAM-G4 onto both PDMS and SU-8 surfaces of the microchannel simultaneously, both amino modified surfaces (i.e. PDMS and SU-8) were treated with a mixture of 1.04 mM EDC, 1.74 mM NHS and PAMAM-G4 in 0.1 M MES solution (2-(4-morpholino)-ethane sulfonic acid, pH 6.0) at 35° C for 2 h. The resulting microchannel was extensively washed by PBS (pH 7.4) at a flow rate of 0.05 mL/h for 10 min, and the obtained microchannel was stored in PBS (pH 7.4) for future use. Three different PAMAM-G4 concentrations (i.e. 0.3, 1.5, and 4.0 μM) were used in this step.

In order to further graft PAMAM-G7 onto both PDMS and SU-8 surfaces inside of the microchannel, both PAMAM-G4 grafted surfaces (i.e. PDMS and SU-8) were treated

with 4.0 μM PAMAM-G7. Same concentrations of NHS, EDC and MES solutions were used at 35° C for 2 h as described above. This was followed by a washing step using PBS (pH 7.4) at a flow rate of 0.05 mL/h for 10 min. Finally, the resulting PAMAM-G7 modified microchannel was stored in PBS (pH 7.4) for future use.

4.3.6 Surface characterizations

In order to evaluate hydrophilicity of different surfaces and to confirm the success of each surface modification step, a goniometer (AST Products Inc., Billerica, MA) was used to characterize surface water contact angles [31]. For each measurement, ~ 10 μL deionized water was dropped onto the surface of interest; after the needle tip withdrawal from the droplet, the water contact angle was measured within 10 sec to minimize water evaporation. Four measurements were performed at different (and random) locations on each sample surface.

A Veeco atomic force microscope (AFM) (Veeco Di Multimode V, Santa Barbara, CA) was used to study the surface morphologies of the surfaces after modifications. Surfaces in each step of modifications were probed in contact mode and subsequently analyzed by a NanoScope software (Veeco, Santa Barbara, CA).

4.3.7 Surface cell non-specific adsorption performances

Surface cell non-specific adsorption performances were evaluated under two different general conditions: performances on flat surfaces and performances inside of microchannels, for both live and dead cells. To achieve this, CCRF-CEM cells, a tumour cell line from human acute lymphoblastic leukemia were used as model cells.

4.3.7.1 Cell non-specific adsorption performance evaluated on flat surfaces

To evaluate cell non-specific adsorption performance on flat surfaces of interest, different flat surfaces – unmodified PDMS surface, PDMS surface modified with APTMS, PDMS surface modified with PAMAM-G4, and PAMAM-G4 modified PDMS surface further modified with PAMAM-G7 were separately incubated with CCRF-CEM cells (1×10^6 cells/mL) (both dead and live) in PBS (pH 7.4) for 1 h. Subsequently the surfaces were carefully and extensively washed with PBS (pH 7.4), and the CCRF-CEM cells that non-specifically adhered to the surfaces were manually counted using Image J Version 1.46 (NIH, Bethesda, MD).

4.3.7.2 Cell non-specific adsorption performance evaluated in microchannels

In order to further evaluate the effects of different surface modifications on non-specific cell adsorption performances inside of microchannels, both live and dead CCRF-CEM cells were used. Briefly, a syringe pump (Harvard Apparatus, Holliston, MA) was used to inject CCRF-CEM cells (1×10^6 cells/mL) in PBS (pH 7.4) into microchannels of interest (i.e. Microchannels 1, 2 and 3) for 1 h at a flow rate of 0.05 mL/h [32]. Subsequently, the microchannels were washed by PBS (pH 7.4) for 10 min. The CCRF-CEM cells that non-specifically adhered to the microchannel walls were manually counted using Image J Version 1.46.

4.4 Results and discussion

4.4.1 Characterization of surface hydrophilicity

Water contact angles of the surfaces with different modifications are shown in Figure 4-2. The water contact angle of the PDMS surface without modification (Surface PDMS) was $120.6^\circ \pm 1.0^\circ$, consistent with previously reported values [33, 34]. As expected, the water contact angle of the PDMS surface reduced to $61.9^\circ \pm 0.8^\circ$ after surface modification with APTMS (Surface APTMS), suggesting that amino groups had been successfully modified onto the PDMS surface [32, 35]. Furthermore, after surface modification with PAMAM-G4 (Surface G4), the surface became even more hydrophilic, registering a water contact angle of $36.5^\circ \pm 0.6^\circ$. It is worthwhile to note that when different concentrations of PAMAM-G4 were used to modify the

APTMS aminated surfaces, higher concentrations of PAMAM-G4 resulted in lower water contact angles for the PAMAM-G4 concentrations investigated (as shown in Appendix B, Figure S2). This is likely because higher PAMAM-G4 concentrations used in the surface modification reactions resulted in higher PAMAM-G4 coverage on the modified surfaces. Finally, the water contact angle of PAMAM-G7 modified PAMAM-G4 surface (Surface G4+G7) was $37.6^{\circ} \pm 1.0^{\circ}$. This value is consistent with the water contact angle of PAMAM-NH₂ modified surfaces in a previous study [36]. It should be mentioned that the water contact angles of G4 and G7+G4 surfaces were not significantly different ($p > 0.05$, student t-test), suggesting that similarly hydrophilic surfaces were obtained in both cases.

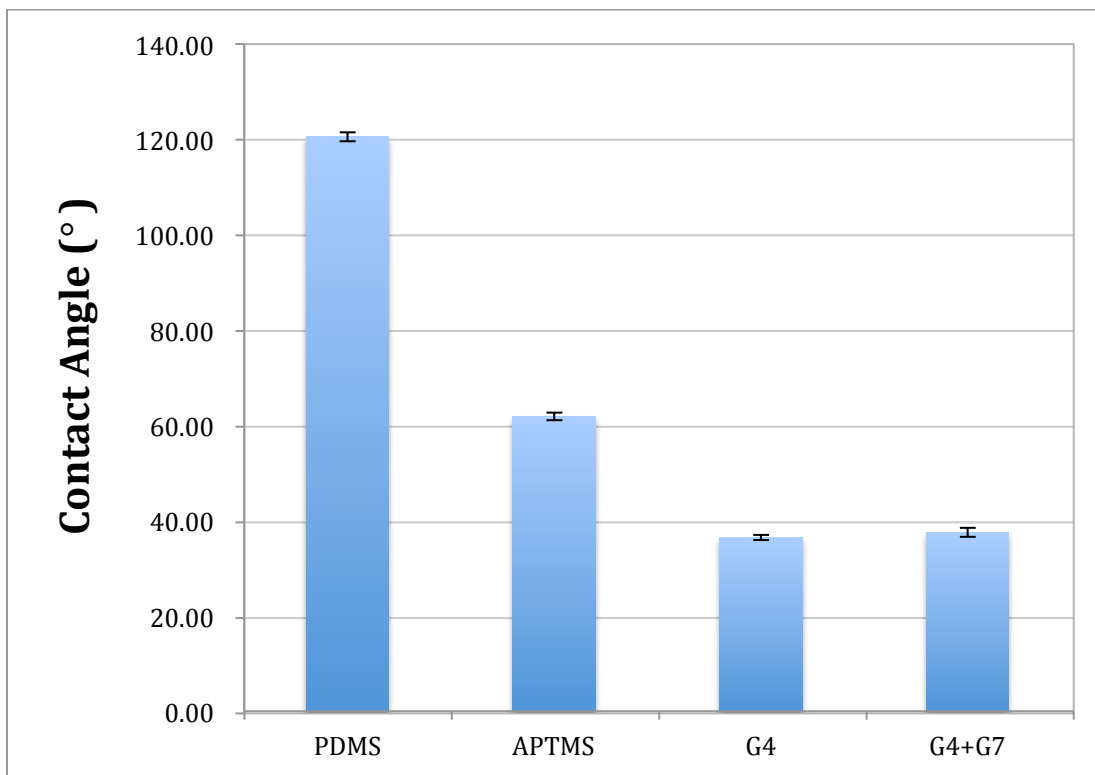


Figure 4-2. Water contact angles of different surfaces of interest. Error bars are standard deviations of the measurements (n=8).

4.4.2 Characterization of surface morphology and PAMAM engraftment

Surface morphology and roughness of modified surfaces were characterized by AFM in contact mode, and the results are shown in Figure 4-3.

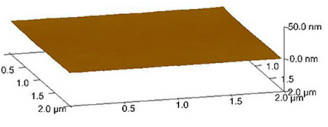
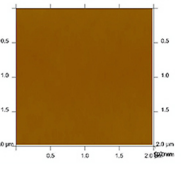
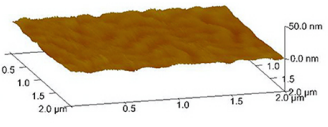
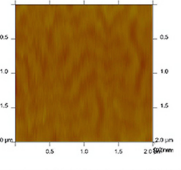
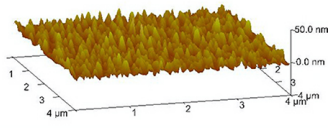
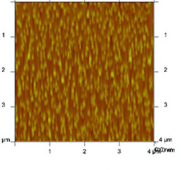
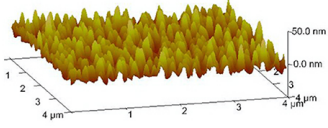
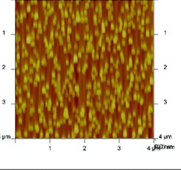
Groups	AFM 3D Image	Vertical View	Roughness
Unmodified PDMS			0.26 ± 0.04
PDMS with APTMS			1.19 ± 0.06
G4			3.62 ± 0.23
G4+G7			5.88 ± 0.33

Figure 4-3. AFM images and roughness (nm) of different surfaces after modifications. The roughness of each surface was calculated to indicate relative surface roughness (n = 4).

As shown in Figure 4-3, surface topographies changed significantly after each surface modification step. It is also evident that the roughness of the surface modified by PAMAM-G7 (i.e. G4+G7) increased dramatically (5.88 ± 0.33) in comparison with that of the surface modified by PAMAM-G4 (i.e. G4) (3.62 ± 0.23) ($p < 0.05$, student t-test). The surface morphology change is likely due to the 3D molecular packing of PAMAM dendrimers on the PDMS surfaces [37]; the dramatic changes of surface roughness also strongly suggest that the PDMS surfaces have been successfully modified by PAMAM dendrimers. Moreover, it is interesting to note that the roughness of PAMAM-G4 modified surfaces increased with increasing PAMAM concentrations used to modify the surfaces (shown in Appendix B, Figure S3). This is consistent with our previous observation [32], which is likely due to the aggregation of PAMAM-G4 dendrimers on corresponding surfaces with increased amount of PAMAM-G4 immobilized on the surfaces [38, 39].

4.4.3 Non-fouling performances of the PDMS surfaces and microchannels modified with different surface modifications

In order to evaluate the effects of different surface modifications on cell non-specific adhesion performances, CCRF-CEM cells were used as model cells in this study. As expected, for dead cell non-fouling performances, the native PDMS surface had the most significant cell non-specific adsorption, followed by APTMS modified surfaces (see Figure 4-4A). Both G4 and G4+G7 modified surfaces showed the least cell non-specific adsorption. This trend is very similar to the trend shown in water contact angle results (see Figure 4-2) regardless of the surface roughness, strongly suggesting that surface hydrophilicity/hydrophobicity is a main factor influencing cell non-specific adhesion while surface roughness is not as important for the case of dead cells. However, for live cells, the trend was completely different. As shown in Figure 4-4A, two aminated surfaces (i.e. APTMS and G4+G7) had the most adherent cells, followed by the native PDMS surface, and subsequently followed by the carboxyl functionalized G4 surface. Evidently this cannot be explained by surface hydrophobicity/hydrophilicity. Instead, this is most likely due to surface charges since it has been reported that dead cells carry no electric charges on the cell surfaces while live cells are negatively charged [40]. This view is supported by the fact that the carboxyl groups on the G4 modified surface are known to be negatively charged at pH 7.4 while the amino groups on both APTMS and G4+G7 modified surfaces are known to be positively charged due to protonation [41]. This result strongly suggests that electric charges of a surface play an important role in the interactions between a surface and live cells. Meanwhile, measurement of zeta

potential of cells could also be used to characterize the difference of electric charge on dead and live cells.

It has been established that there is an optimal surface hydrophilicity for non-specific cell adhesions, and the water contact angle of the optimal surface for live cell adhesion is reported to be between 60° and 80° [42-45]. This phenomenon has been attributed to the preferential adsorption of cell-adhesive proteins from the cell culture media onto the moderately hydrophilic surfaces [44, 45]. Interestingly, it is noted that the non-specific cell adhesion results from the current study (for both live and dead cells) did not follow the same trend to show optimal cell adhesions on “moderately” hydrophilic surfaces. This is likely due to the fact that the current cell adhesion study was conducted in PBS (pH 7.4) without any dissolved proteins. A closer analysis of the data showed that the amount of immobilized amino groups on the APTMS modified surfaces was greater than that on the G7 modified (i.e. G4+G7) surfaces. This likely explains the observation that for live cells, APTMS modified surfaces had more non-specific cell adhesions than G4+G7 modified surfaces (see Figure 4-4) since it has been reported that the number of non-specifically adsorbed cells increases with increasing cationic charge densities on the surfaces [46, 47]. It should be noted that although the roughness of G4 modified surface is much greater than those of both native PDMS and APTMS modified surfaces, it had the least adherent cells, suggesting that roughness is not as an important factor as electric charge in the case of live cells. It is also interesting to note from Figure 4-4A that the native PDMS surface adsorbed less live cells than it did dead cells. This is likely

because that live cells are more hydrophilic than dead cells, resulting less hydrophobic interactions with PDMS surfaces [48].

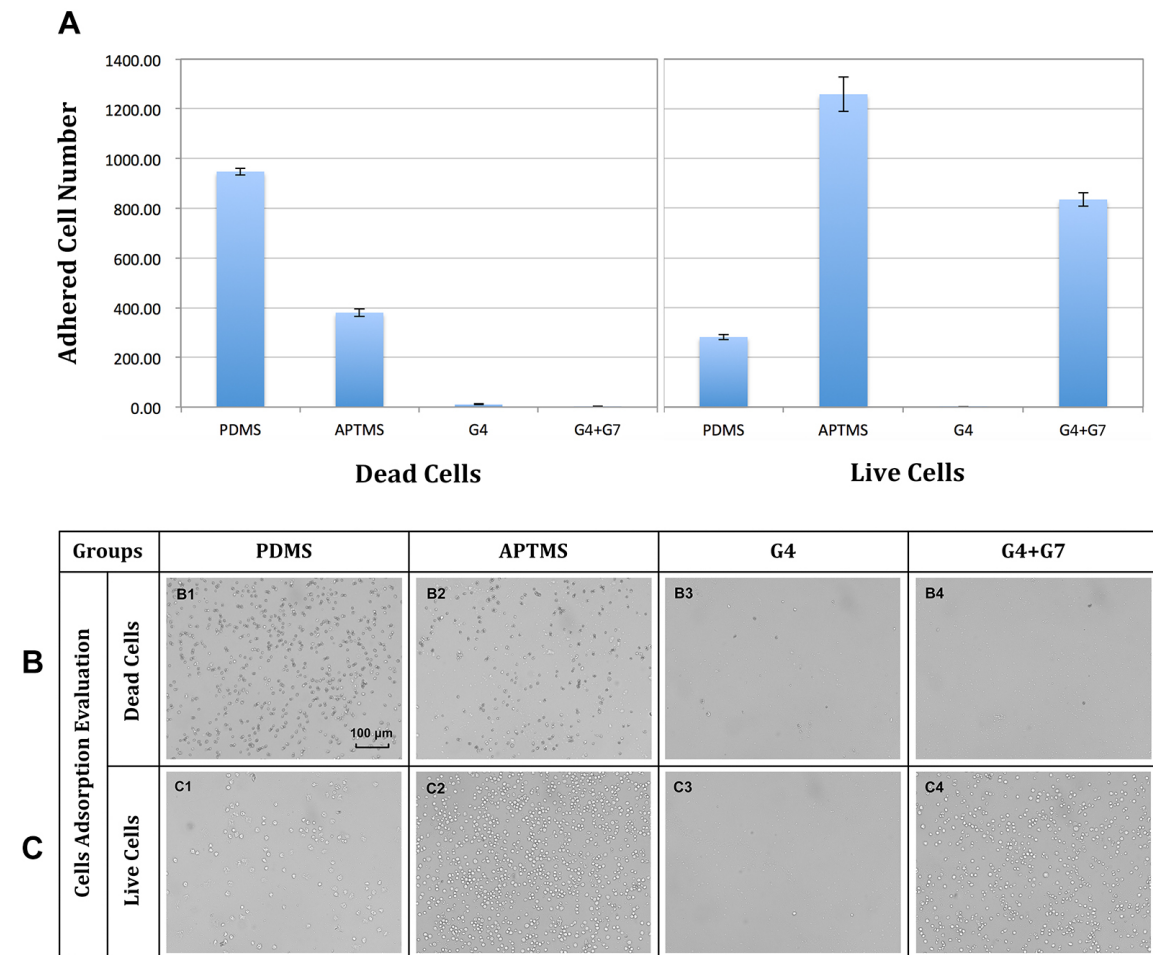


Figure 4-4. Non-fouling performances of PDMS surfaces with different surface modifications. (A) bar plot demonstrating cell (both live and dead cells) non-specific adhesions to different surfaces. Error bars are standard deviations of the measurements (n=4). (B) micrographs showing cell non-specific adhesions to different surfaces as evaluated by dead CCRF-CEM cells: (B1) native PDMS surface,

(B2) PDMS surface modified with APTMS, (B3) PDMS surface modified by PAMAM-G4 dendrimer, and (B4) PDMS surface further modified by PAMAM-G7 dendrimer after modification by PAMAM-G4 dendrimer. (C) micrographs showing cell non-specific adhesions to different surfaces as evaluated by live CCRF-CEM cells: (C1) native PDMS surface, (C2) PDMS surface modified with APTMS, (C3) PDMS surface modified by PAMAM-G4 dendrimer, and (C4) PDMS surface further modified by PAMAM-G7 dendrimer after modification by PAMAM-G4 dendrimer.

Similarly, non-fouling performances of microfluidic channels with different surface modifications were also evaluated, and the results were consistent with those obtained from flat surface studies. As shown in Figure 4-5, for dead cells, APTMS modified surface adsorbed significantly more cells than either G4 or G4+G7 surfaces, regardless of their surface roughness, suggesting that hydrophilicity/hydrophobicity played a major role in cell non-specific adhesion performances. For live cells, it is clear that microchannel surfaces with positive charges, such as APTMS and G4+G7, adsorbed much more live cells than surfaces with negative charges, such as G4, regardless of their surface hydrophilicity/hydrophobicity, suggesting that interactions between live cells and microfluidic surfaces are mainly determined by electric charges. Similar to results on flat surfaces, surface roughness did not seem to be as important as the other two factors under the experimental conditions used in this study.

Given the results presented above, it is clear that in designing a microfluidic device for high sensitivity detection applications, one should consider not only the surface properties (i.e. hydrophilicity/hydrophobicity) of the microfluidic device but also the electric charge properties of the targets as well as those of the non-targets. The results also suggest that when selecting model particles (e.g. formalin killed cells, polystyrene microbeads or live cells) to simulate interactions between particles and microchannel surfaces, more considerations should be given to both hydrophilicity/hydrophobicity interactions and electric charge interactions between microfluidic channel surfaces and the particles.

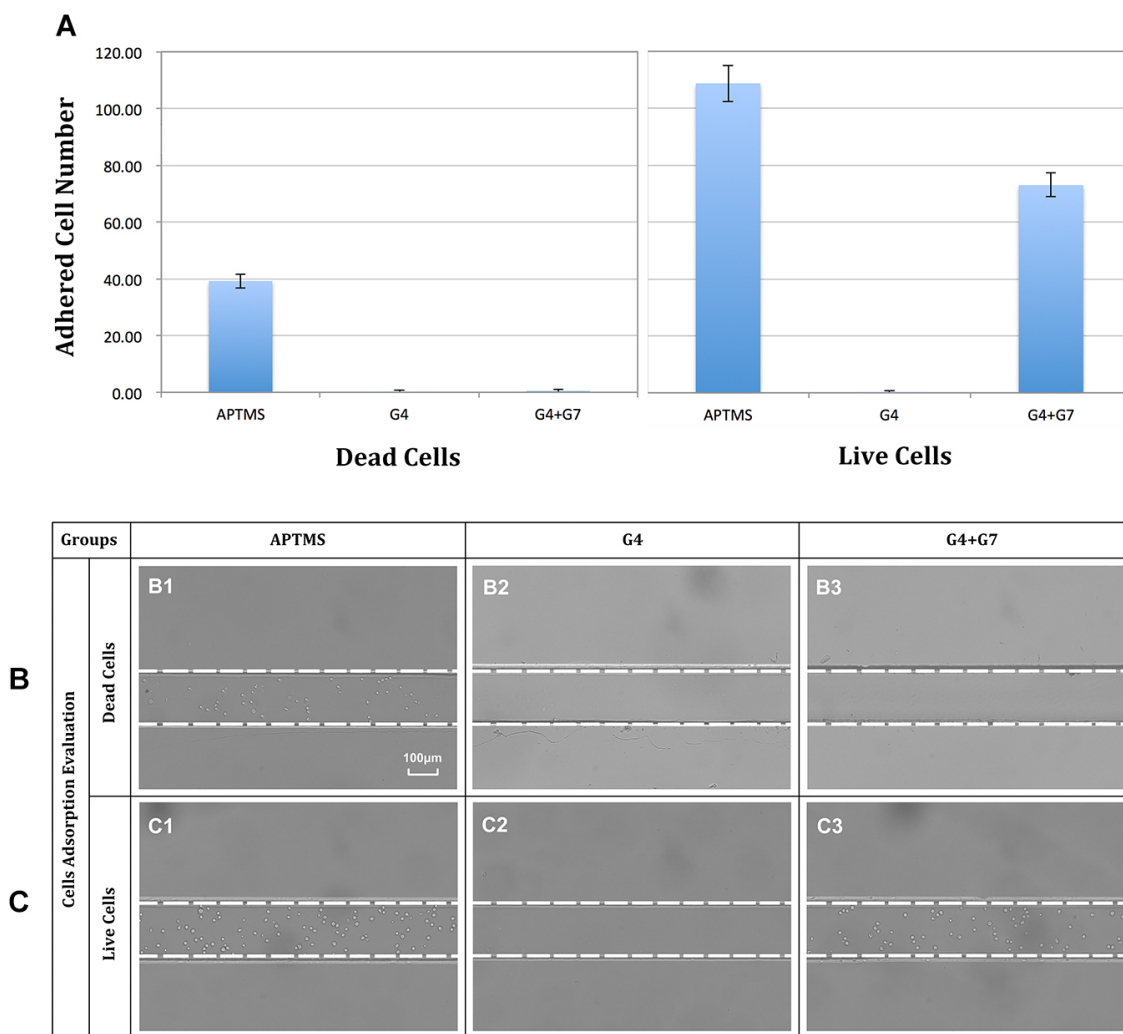


Figure 4-5. Non-fouling performance of microfluidic channels with different surface modifications. (A) bar plot demonstrating cell non-specific adhesions to different surfaces in microchannels. Error bars are standard deviations of the measurements (n=4). (B) micrographs of cell non-specific adhesions in different microchannels as evaluated by dead CCRF-CEM cells: (B1) microchannel functionalized by amino groups, (B2) microchannel modified by PAMAM-G4 dendrimer, and (B3)

microchannel further modified by PAMAM-G7 dendrimer after modification by PAMAM-G4 dendrimer. (C) micrographs of cell non-specific adhesions in different microchannels as evaluated by live CCRF-CEM cells: (C1) microchannel functionalized by amino groups, (C2) microchannel modified by PAMAM-G4 dendrimer, and (C3) microchannel further modified by PAMAM-G7 dendrimer after modification by PAMAM-G4 dendrimer.

4.5 Conclusions

Using both live and dead CCRF-CEM cells as model cells for CTCs, we 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 while 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. We also present a new surface modification strategy that takes surface charges and hydrophilicity/hydrophobicity into consideration to prepare lab-on-a-chip microfluidic systems for high sensitivity biosensing and detection applications by decreasing its background signals. It is also worthwhile to note that interactions between microfluidic channel surfaces and cells (i.e. live and dead) are significantly different, and therefore should be carefully considered when choosing model cells for any particular lab-on-a-chip applications.

4.6 References

- [1] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, *CA: a cancer journal for clinicians*, 61 (2011) 69-90.
- [2] I.J. Fidler, The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited, *Nature Reviews Cancer*, 3 (2003) 453-458.
- [3] M.S. Wicha, D.F. Hayes, Circulating tumor cells: not all detected cells are bad and not all bad cells are detected, *Journal of Clinical Oncology*, 29 (2011) 1508-1511.
- [4] P.S. Steeg, Tumor metastasis: mechanistic insights and clinical challenges, *Nature medicine*, 12 (2006) 895-904.
- [5] G.P. Gupta, J. Massagué, Cancer metastasis: building a framework, *Cell*, 127 (2006) 679-695.
- [6] M. Cristofanilli, G.T. Budd, M.J. Ellis, A. Stopeck, J. Matera, M.C. Miller, J.M. Reuben, G.V. Doyle, W.J. Allard, L.W. Terstappen, Circulating tumor cells, disease progression, and survival in metastatic breast cancer, *New England Journal of Medicine*, 351 (2004) 781-791.
- [7] D.C. Danila, M. Fleisher, H.I. Scher, Circulating tumor cells as biomarkers in prostate cancer, *Clinical Cancer Research*, 17 (2011) 3903-3912.
- [8] B. Hong, Y. Zu, Detecting circulating tumor cells: current challenges and new trends, *Theranostics*, 3 (2013) 377-394.
- [9] E. Racila, D. Euhus, A.J. Weiss, C. Rao, J. McConnell, L.W. Terstappen, J.W. Uhr, Detection and characterization of carcinoma cells in the blood, *Proceedings of the National Academy of Sciences*, 95 (1998) 4589-4594.
- [10] P. Pinzani, B. Salvadori, L. Simi, S. Bianchi, V. Distanti, L. Cataliotti, M. Pazzagli, C. Orlando, Isolation by size of epithelial tumor cells in peripheral blood of patients with breast cancer: correlation with real-time reverse transcriptase-polymerase chain reaction results and feasibility of molecular analysis by laser microdissection, *Human pathology*, 37 (2006) 711-718.
- [11] W.J. Allard, J. Matera, M.C. Miller, M. Repollet, M.C. Connelly, C. Rao, A.G. Tibbe, J.W. Uhr, L.W. Terstappen, Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases, *Clinical Cancer Research*, 10 (2004) 6897-6904.
- [12] W. Liu, X. Zhou, D. Xing, Rapid and reliable microRNA detection by stacking hybridization on electrochemiluminescent chip system, *Biosensors and Bioelectronics*, 58 (2014) 388-394.
- [13] J. Horak, C. Dincer, H. Bakirci, G. Urban, Sensitive, rapid and quantitative detection of substance P in serum samples using an integrated microfluidic immunochip, *Biosensors and Bioelectronics*, 58 (2014) 186-192.
- [14] J.A. Phillips, Y. Xu, Z. Xia, Z.H. Fan, W. Tan, Enrichment of cancer cells using aptamers immobilized on a microfluidic channel, *Analytical chemistry*, 81 (2008) 1033-1039.

- [15] Y. Xu, J.A. Phillips, J. Yan, Q. Li, Z.H. Fan, W. Tan, Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells, *Analytical chemistry*, 81 (2009) 7436-7442.
- [16] W. Sheng, T. Chen, R. Kamath, X. Xiong, W. Tan, Z.H. Fan, Aptamer-enabled efficient isolation of cancer cells from whole blood using a microfluidic device, *Analytical chemistry*, 84 (2012) 4199-4206.
- [17] J.-Y. Chen, W.-S. Tsai, H.-J. Shao, J.-C. Wu, J.-M. Lai, S.-H. Lu, T.-F. Hung, C.-T. Yang, L.-C. Wu, J.-S. Chen, Sensitive and Specific Biomimetic Lipid Coated Microfluidics to Isolate Viable Circulating Tumor Cells and Microemboli for Cancer Detection, *PloS one*, 11 (2016) e0149633.
- [18] H. Zhang, Z. Jia, C. Wu, L. Zang, G. Yang, Z. Chen, B. Tang, In vivo capture of circulating tumor cells based on transfusion with a vein indwelling needle, *ACS applied materials & interfaces*, 7 (2015) 20477-20484.
- [19] M. Yu, S. Stott, M. Toner, S. Maheswaran, D.A. Haber, Circulating tumor cells: approaches to isolation and characterization, *The Journal of cell biology*, 192 (2011) 373-382.
- [20] J. Hou, Q. Shi, W. Ye, Q. Fan, H. Shi, S.-C. Wong, X. Xu, J. Yin, A novel hydrophilic polymer-brush pattern for site-specific capture of blood cells from whole blood, *Chemical Communications*, 51 (2015) 4200-4203.
- [21] N. Sun, J. Wang, L. Ji, S. Hong, J. Dong, Y. Guo, K. Zhang, R. Pei, A Cellular Compatible Chitosan Nanoparticle Surface for Isolation and In Situ Culture of Rare Number CTCs, *Small*, 11 (2015) 5444-5451.
- [22] R.A. Hartvig, M. van de Weert, J. Østergaard, L. Jorgensen, H. Jensen, Protein adsorption at charged surfaces: the role of electrostatic interactions and interfacial charge regulation, *Langmuir*, 27 (2011) 2634-2643.
- [23] W.-I. Wu, K.N. Sask, J.L. Brash, P.R. Selvaganapathy, Polyurethane-based microfluidic devices for blood contacting applications, *Lab on a Chip*, 12 (2012) 960-970.
- [24] J. Thies, J. Qiu, DSM Non-Biofouling Coatings: A stealth approach to the fighting biofilms, *Journal of Biomedical Materials Research Part A*, 91A (2009) 824-826.
- [25] W. Xu, Y. Wang, S. Liu, J. Yu, H. Wang, J. Huang, A novel sandwich-type electrochemical aptasensor for sensitive detection of kanamycin based on GR-PANI and PAMAM-Au nanocomposites, *New Journal of Chemistry*, 38 (2014) 4931-4937.
- [26] A. Asefnezhad, N. Montazeri, The relationship between cellular adhesion and surface roughness in polystyrene modified by microwave plasma radiation, *International journal of nanomedicine*, 6 (2011) 631-639.
- [27] S. Prakash, T.M. Long, J. Wan, J.S. Moore, M.A. Shannon, Electroosmotic Flow in "Click" Surface Modified Microfluidic Channels, *ASME 4th International Conference on Nanochannels, Microchannels, and Minichannels*, American Society of Mechanical Engineers, 2006, pp. 249-255.
- [28] P.Y. Yeh, Z. Zhang, M. Lin, X. Cao, Nonfouling hydrophilic poly (ethylene glycol) engraftment strategy for PDMS/SU-8 heterogeneous microfluidic devices, *Langmuir*, 28 (2012) 16227-16236.
- [29] E. Ouellet, C.W.T. Yang, T. Lin, L.L. Yang, E.T. Lagally, Novel carboxyl-amine bonding methods for poly (dimethylsiloxane)-based devices, *Langmuir*, 26 (2010) 11609-11614.

- [30] Z. Zhang, P. Zhao, G. Xiao, The fabrication of polymer microfluidic devices using a solid-to-solid interfacial polyaddition, *Polymer*, 50 (2009) 5358-5361.
- [31] Y. Qin, P. Yeh, X. Hao, X. Cao, Developing an ultra non-fouling SU-8 and PDMS hybrid microfluidic device by poly (amidoamine) engraftment, *Colloids and Surfaces B: Biointerfaces*, 127 (2015) 247-255.
- [32] F. Walther, P. Davydovskaya, S. Zürcher, M. Kaiser, H. Herberg, A.M. Gigler, R.W. Stark, Stability of the hydrophilic behavior of oxygen plasma activated SU-8, *Journal of Micromechanics and Microengineering*, 17 (2007) 524.
- [33] S. Prabakaran, S. Jayaraman, D.H. Minh, S.K. Sinha, The role of functional end groups of perfluoropolyether (Z-dol and Z-03) lubricants in augmenting the tribology of SU-8 composites, *Tribology Letters*, 56 (2014) 423-434.
- [34] G. Li, L. Shen, Y. Luo, S. Zhang, The effect of silver-PAMAM dendrimer nanocomposites on the performance of PVDF membranes, *Desalination*, 338 (2014) 115-120.
- [35] F. Rupp, L. Scheideler, J. Geis - Gerstorfer, Effect of heterogenic surfaces on contact angle hysteresis: dynamic contact angle analysis in material sciences, *Chemical engineering & technology*, 25 (2002) 877-882.
- [36] V. Katur, M. Eichler, E. Deigle, C. Stage, P. Karageorgiev, J. Geis-Gerstorfer, G. Schmalz, S. Ruhl, F. Rupp, R. Müller, Surface-immobilized PAMAM-dendrimers modified with cationic or anionic terminal functions: Physicochemical surface properties and conformational changes after application of liquid interface stress, *Journal of colloid and interface science*, 366 (2012) 179-190.
- [37] M.J. Jasmine, E. Prasad, Fractal growth of PAMAM dendrimer aggregates and its impact on the intrinsic emission properties, *The Journal of Physical Chemistry B*, 114 (2010) 7735-7742.
- [38] H.A. Essawy, H.A. Mohamed, Poly (amidoamine) dendritic structures, bearing different end groups, as adhesion promoters for urea-formaldehyde wood adhesive system, *Journal of Applied Polymer Science*, 119 (2011) 760-767.
- [39] A. Deepu, V. Sai, S. Mukherji, Simple surface modification techniques for immobilization of biomolecules on SU-8, *Journal of Materials Science: Materials in Medicine*, 20 (2009) 25-28.
- [40] R.A. Lawton, C.R. Price, A.F. Runge, W.J. Doherty, S.S. Saavedra, Air plasma treatment of submicron thick PDMS polymer films: effect of oxidation time and storage conditions, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 253 (2005) 213-215.
- [41] M. Luo, A dietotherapy granule, Patents CN100464784 C, 2009.
- [42] Y. Tamada, Y. Ikada, Effect of preadsorbed proteins on cell adhesion to polymer surfaces, *Journal of colloid and interface science*, 155 (1993) 334-339.
- [43] E.A. Vogler, Water and the acute biological response to surfaces, *Journal of Biomaterials Science, Polymer Edition*, 10 (1999) 1015-1045.
- [44] D.P. Dowling, I.S. Miller, M. Ardhaoui, W.M. Gallagher, Effect of surface wettability and topography on the adhesion of osteosarcoma cells on plasma-modified polystyrene, *Journal of biomaterials applications*, 26 (2010) 327-347.

- [45] J.H. Lee, G. Khang, J.W. Lee, H.B. Lee, Interaction of different types of cells on polymer surfaces with wettability gradient, *Journal of colloid and interface science*, 205 (1998) 323-330.
- [46] S. Kim, A.E. English, K.D. Kihm, Surface elasticity and charge concentration-dependent endothelial cell attachment to copolymer polyelectrolyte hydrogel, *Acta biomaterialia*, 5 (2009) 144-151.
- [47] G.B. Schneider, A. English, M. Abraham, R. Zaharias, C. Stanford, J. Keller, The effect of hydrogel charge density on cell attachment, *Biomaterials*, 25 (2004) 3023-3028.
- [48] G. Jesse, G. Shana, L. Tony, G. Zara, *Membrane structure*, Davidson college, 2002, Chapter 6.

Chapter 5 Effects of aptamer spacer length on circulating tumour cell detection in microfluidic systems

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(Preparing for publication)

5.1 Abstract

Accurate secondary structure formation of aptamers is a challenge in sensitive detections using aptamers as biomarkers, such as detecting circulating tumour cells in microfluidic systems. In this report, we present a new strategy to study the effect made by spacer length of aptamer on cell capture performance in a PDMS/SU-8 microfluidic system. To achieve this, microchannel surfaces were modified by aptamers with two, five, ten, or twenty thymine bases as spacer that rendered different space for aptamers to form their secondary structures. Water contact angle, RNAstructure simulation and microscopy were used to characterize and confirm surface modifications and aptamer structure formations, and the cell capture performance of the resulting surfaces was tested using live CCRF-CEM cancer cells. Our results show that ten thymine bases spacer has the best length as a spacer for sgc8 aptamer for CCRF-CEM cell capture under the experimental conditions used in this study. Spacers shorter than ten thymine bases result in lack of space for aptamers that limiting their ability to capture target cells. When the spacers of the aptamers are in an excessive length, the secondary structure formation of partial aptamers will change and lose their capture ability to capture target cells. We also present a surface modification strategy by using highly branched polymer, PAMAM dendrimers that can not only provide large loading capacity for aptamers by their multi-functional sites but also decrease background signals by their hydrophilic surface property, which is significantly important for preparing lab-on-a-chip microfluidic systems for high sensitivity biosensing and detection applications.

5.2 Introduction

Cancer is the second leading cause of death globally, which is responsible for nearly 30% of all death [1]. It has been reported that greater than 90% of cancer patients died from cancer metastasis [2, 3]. During the process of cancer, cancer cells detach from the primary tumour, spread to surrounding tissues and enter bloodstream, which is called circulating tumour cells (CTCs) [4-6]. In recent years, CTCs have attracted growing research interest in the field of cancer diagnosis and cancer cell detection. This is due to CTCs can be potentially applied as indicators for early stage cancer detection and diagnosis, which is significantly important for early personalized therapeutic treatments and real-time monitoring to improve the survival rate of cancer patients [7-9].

The general detection methods of CTCs can be sorted into detections based on bench-top instruments and detections based on microfluidic systems [10]. To date, detection methods based on bench-top instruments are the major methods for CTCs detections, such as CellSearch system [11], flow cytometers [12] and isolation by the size of epithelial tumour cells [13]. Compared with bench-top instruments, CTC detection methods based on microfluidic systems show many advantages, such as high detection sensitivity [14], relatively low cost for fabrication, and low sample volume requirement [15]. Aptamers are single strands of nucleic acids that, like antibodies, bind molecular targets with high affinity and specificity. Different with

the reaction between antibodies and antigens, aptamers structurally conform to bind to their targets by their secondary structures. Advantages of aptamers include *in vitro* selection, chemical synthesis, reproducible and specific chemical modifications, and good chemical and thermal stability [16, 17]. In the past decade, aptamer functionalized microfluidic systems with applications in biological and chemical analysis have shown their importance in biochemistry, biology and diagnostics [18, 19]. For example, a graphene oxide based Forster resonance energy transfer (FRET) aptasensing microfluidic device has been used in a visual and high-throughput detection of CTCs (e.g. CCRF-CEM, T-leukemia cell line) [20]. Moreover, a poly(dimethylsiloxane) (PDMS) microfluidic device has been made for efficient CCRF-CEM cells isolation from whole blood by surface modification with aptamers [19]. Aptamers are originally selected from a large oligonucleotide library based on their recognitions to target ligands in liquid solutions [21]. When introducing aptamers to solid surfaces, spacers are needed to help the immobilized aptamers overcome steric interference in order to have the freedom to form their secondary structures [22, 23]. Generally, a polyethylene glycol (PEG) or an oligo-T (thymine) spacer is used as the spacer for an aptamer [24]. Although aptamers have been widely applied in microfluidic systems, binding capacity, as well as capture performance of the designed aptamer-spacer molecules are still challenges in this area, which are significantly important for their applications in CTC detections in microfluidic devices. In order to improve capture performance of aptamers in microfluidic systems, approaches such as surface plasmon resonance and rolling circle amplification have been studied and showed their improvements on capturing

performance in aptamer-based microfluidic systems [25, 26]. However, it should be noted that the spacer of an aptamer is also an important factor that affecting capture performance of the aptamer [23]. Brett Waybrant has investigated how the length and hydrophobicity of the spacer molecule inserted between the tail and the aptamer head group, affect the binding, structure, and self-assembly properties of the aptamer-amphiphile [22]. In this study, researchers synthesised aptamer-amphiphiles with either no spacer, polyethylene glycol (PEG₄, PEG₈, PEG₂₄), alkyl (C₁₂ and C₂₄), or oligonucleotide (T₁₀ and T₅: 10 and 5 thymine, and A₁₀ adenine) as spacers. It has been showed that the hydrophobic alkyl spacers resulted in the greatest loss of affinity, and the hydrophilic PEG spacers improved amphiphile affinity but did not restore it to that of the free aptamer. Interestingly, oligonucleotide spacers produced the highest affinity amphiphiles. Nucleotide composition did not affect affinity and the T₁₀ and A₁₀ spacers had equal affinity. However, to the best of our knowledge, there is no study investigating the effect of the length of oligonucleotide spacers on the target capture performance of aptamers when the length is excessive long. This is significantly important because the oligonucleotide spacer can form base pairing with linked aptamer or bases from another aptamer, which is significantly different with aptamer spacers such as PEG and alkyl spacers.

In this study, PAMAM dendrimer was used to modify the surfaces within the PDMS and SU-8 heterogeneous microfluidic channels to provide high loading capacity for aptamers. Moreover, carboxyl functionalized PAMAM dendrimer with high

hydrophilicity could protect the surface from non-specific adsorption at the same time. Aptamers with four different lengths of spacers (i.e. Aptamer-T2, Aptamer-T5, Aptamer-T10 and Aptamer-T20), as well as a control DNA sequence (i.e. Random-T10) were used to investigate the effect of spacer length on target cell capture performance. Our results show that ten thymine bases spacer has the best length as a spacer for sgc8 aptamer for CCRF-CEM cell capture under the experimental conditions used in this study. Spacers shorter than ten thymine bases will result in lack of space for aptamers that limiting their ability to capture target cells. However, when the spacers of the aptamers are in an excessive length, the secondary structure formation of partial aptamers will change and lose their capture ability to capture target cells. Understanding how the length of spacers making influence on the secondary structure of aptamers is very important because secondary structure of an aptamer directly affecting its recognition and capture performance, especially in applications where sensitivities are critically important such as in CTC detections.

5.3 Experimental

5.3.1 Materials

Sylgard 184 PDMS kit was purchased from Dow Corning (Midland, MI). (3-Aminopropyl)-trimethoxysilane (313251000, APTMS) and *N*-hydroxysuccinimide (157270250, NHS) were obtained from Thermo Scientific Inc. (Rockford, IL). SU-8 2050 negative photoresist was purchased from MicroChem (Newton, MA).

Ethylenediamine anhydrous (E479-500, EDA) and toluene (T324-4) were obtained from Fisher Scientific Inc. (Ottawa, ON). PAMAM dendrimer solution (ethylenediamine core, generation 7, succinamic acid surface) (i.e. PAMAM-COOH) was purchased from Dendritech (Midland, MI). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (A10807, EDC) was obtained from Alfa Aesar (Ward Hill, MA). Furthermore, CCRF-CEM cells (CCL-119, human acute lymphoblastic leukemia, T cell line) and Ramos cells (CRL-1596, human Burkitt's lymphoma, B-cell) were obtained from American Type Culture Collection (ATCC) (Manassas, VA). In addition, 3' end amine capped aptamers were purchased from Integrated DNA Technologies (IDT) (Coralville, IA). Aptamer sequences are as follows: aptamer sgc8 with two thymine bases as its spacer (i.e. Aptamer-T2, spacer is shown underlined on the 3' end), 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT T-NH₂-3'; aptamer sgc8 with five thymine bases as its spacer (i.e. Aptamer-T5), 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT-NH₂-3'; aptamer sgc8 with ten thymine bases as its spacer (i.e. Aptamer-T10), 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTT TTT-NH₂-3'; aptamer sgc8 with twenty thymine bases as its spacer (i.e. Aptamer-T20), 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GAT TTT TTT TTT TTT TTT TTT-NH₂-3' and a random single stranded DNA (same number of bases with aptamer sgc8, as a control group) with ten thymine bases as its spacer (i.e. Random-T10), ATT ACC TCT AAA TCA CTG CTC TGT AAC ATG GTC GCG CTA GGT TTT TTT TTT-NH₂-3'. All other chemicals were purchased from Sigma-Aldrich (Oakville, ON) and used as received unless indicated otherwise.

5.3.2 General approach

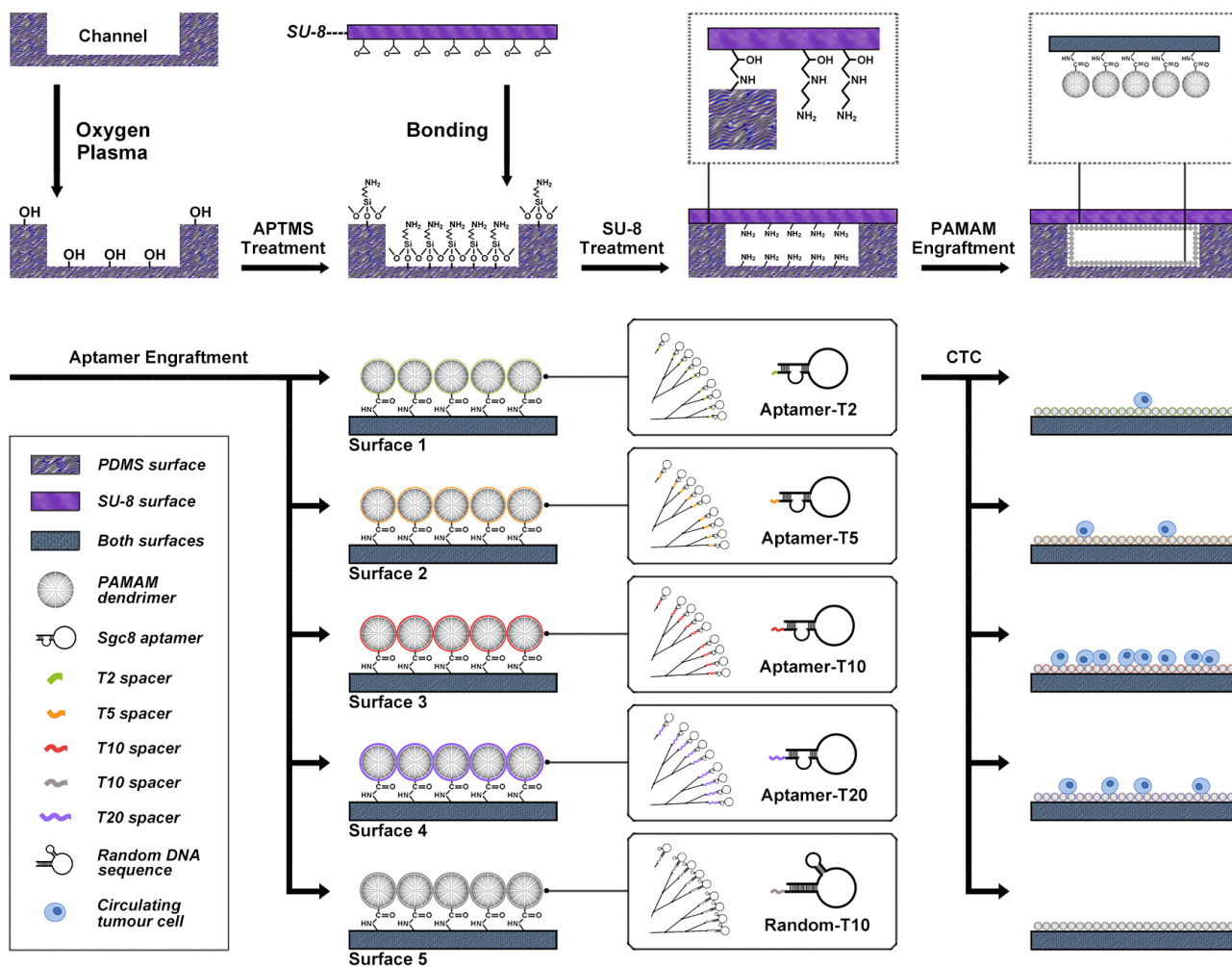


Figure 5-1. A schematic drawing to outline overall experimental procedures for microfluidic device fabrications, surface modifications and CTC captures.

As shown in Figure 5-1, the original PDMS surface is initially activated by oxygen plasma and subsequently aminated by APTMS through silanol condensations [27]. The resulting PDMS surface is then combined with original SU-8 surface through reactions between amino groups and epoxy groups on the aminated PDMS surface and original SU-8 surface, respectively, to seal the microfluidic channel. The free SU-8 surface in the resulting sealed microchannel is further aminated by EDA treatment. Subsequently, PAMAM dendrimers are simultaneously immobilized onto both aminated SU-8 and PDMS surfaces inside of the microchannel through reactions between carboxyl groups of the PAMAM dendrimers and amino functional groups on both modified SU-8 and PDMS surfaces. Finally, amine-functionalized aptamers with different spacer lengths are tethered onto the surfaces of the microfluidic channels via the PAMAM templates through reactions between the carboxyl groups of the PAMAM dendrimers and the amino groups of the 3' ends of the amine capped aptamers. Aptamer-T2, Aptamer-T5, Aptamer-T10, Aptamer-T20 and Random-T10 are used to obtain Surface 1, Surface 2, Surface3, Surface 4 and Surface 5, respectively. Finally, CCRF-CEM cells, which can be specifically recognized by sgc8 aptamer, are used as model cells for circulating tumour cells to evaluate CTC capturing properties of the resulting surfaces of interest [28, 29].

5.3.3 PDMS microchannel preparation

A typical soft lithography process was used to prepare the PDMS surfaces. Briefly, silicone elastomer base Sylgard 184 was manually mixed with its curing agent for 6

min in a 10 (base): 1 (curing agent) ratio by weight; the mixture was poured carefully onto a microchannel-patterned SU-8 master. In order to cure the polymer, the mixture was degassed for 40 min and baked at 110 ° C for 1.5 h. Finally, the cured PDMS with microchannel patterns was carefully peeled off from the SU-8 master. Flat PDMS surfaces without any microchannel patterns were prepared in the same procedure except that the PDMS mixture was poured onto a bare silicon wafer (4 inch) instead of a patterned SU-8 master.

5.3.4 Aminations of PDMS and SU-8 surfaces

A silane coupling method was employed to introduce amino groups onto the PDMS surface as previously reported [30, 31]. Briefly, original PDMS surface was first treated with oxygen plasma by a plasma cleaner (Anatech Ltd, SP100, Battle Creek, MI) at 119 W, 100 mTorr for 10 s. Subsequently, the resulting PDMS surface was immediately treated with APTMS solution (0.05 wt.% in toluene) for 20 s to introduce amino groups onto the PDMS surface through silanol condensation. Finally, the resulting PDMS surface was air-dried.

To aminate the SU-8 surface, EDA ethanol solution (20 wt.% in anhydrous alcohol) was used to treat the SU-8 surface for 1 h, and the resulting surface was subsequently dried at 70° C for another hour. This allowed the amino groups on one end of the bifunctional EDA molecules to react with the epoxy groups on the SU-8 surface while leaving the other end available to enable further PAMAM engraftment.

5.3.5 PDMS/SU-8 heterogeneous device fabrication

In order to seal a PDMS/SU-8 heterogeneous microfluidic device, the APTMS treated PDMS surface was first combined with an original SU-8 surface under 1.8×10^{-2} MPa pressure at 120° C for 40 min. Stainless steel tubes were subsequently inserted into the terminals of the microfluidic channels and secured by pressure sealing at 140° C for another 40 min. Finally, SU-8 surface in the resulting heterogeneous microfluidic channels ($W \times D$, 150 $\mu\text{m} \times 60 \mu\text{m}$) was further aminated by injecting EDA solution (20 wt.% in anhydrous alcohol) into the packaged microfluidic channel at a flow rate of 0.05 mL/h for 1 h and then dried at 70° C for another hour.

In order to conjugate PAMAM dendrimer onto both PDMS and SU-8 surfaces in the microchannel at the same time, both aminated surfaces (i.e. PDMS and SU-8 surfaces) were incubated with a mixture of 4.0 μM PAMAM dendrimer, 1.04 mM EDC and 1.74 mM NHS in 0.1 M MES solution (2-(4-morpholino)-ethane sulfonic acid, pH 6.0) at 37 ° C and at a flow rate of 0.05 mL/h for 2 h. Subsequently, the resulting microchannel was washed extensively using PBS (pH 7.4) at the same flow rate for 10 min. Finally, the resulting PAMAM modified microchannel was stored in PBS (pH 7.4) for future use.

In order to further immobilize aptamers onto both PDMS and the SU-8 surfaces in the microchannel, both PAMAM dendrimer grafted surfaces (i.e. PDMS and SU-8) were washed with MES solution (pH 6.0) at a flow rate of 0.05 mL/h for 10min. Subsequently, a mixture of 5.22 mM NHS and 2.60×10^2 mM EDC in 0.1 M MES solution (pH 6.0) was injected into the microchannel at a flow rate of 0.05 mL/h under room temperature for 1h. This was followed by a washing step with nuclease free water at a flow rate of 0.05 mL/h for 10 min and air-dried. Finally, 10 μ M sgc8 aptamers or random single stranded DNA (i.e. scrambled aptamer sequence) in IDTE buffer was injected into the PAMAM modified microchannel at 37 ° C and a flow rate of 0.05 mL/h for 1h. The aptamers used in the microchannel were capped with amino groups at 3' end, allowing the conjugation of aptamers with the carboxyl functionalized PAMAM dendrimers in a well-controlled manner. Four different aptamers (i.e. Aptamer-T2, Aptamer-T5, Aptamer-T10 and Aptamer-T20) and one random single stranded DNA (i.e. Random-T10) were used in the surface modifications.

5.3.6 Characterizations and simulations

In order to confirm surface modifications in each step, a goniometer (AST Products Inc., Billerica, MA) was used to measure water contact angles of surfaces of interest [32]. Briefly, 10 μ L of deionized water was used in each measurement; to minimize water evaporation, measurements were taken within 10 s after needle tip withdrawal from the water droplet. For each sample, four measurements were

taken at random and different locations on the sample surface, and the averaged values were reported [32].

In order to investigate conformation changes of different aptamers with spacers of different lengths, software RNAstructure (Version 5.2) (<https://rna.urmc.rochester.edu/RNAstructure.html>) was used to simulate the secondary structures of the sequences of interest. RNAstructure is a complete simulation package for DNA or RNA secondary structure prediction and analysis. By calculating the lowest free energy, the simulation was used to predict the most likely secondary structures of each DNA sequences of interest [33, 34]. More specifically, for a typical simulation, the sequence of an sDNA of interest was initially imported into the software. The parameters of temperature, maximum percentage energy difference and maximum number of structures were set to be 310.15 K (i.e. 37 ° C), 50% and 5, respectively. Subsequently, the sequence was folded into different secondary structures and the free energies of these potential secondary structures were calculated. Finally, the secondary structure corresponding to the lowest free energy was presented as the predicted secondary structure of the DNA sequence of interest.

5.3.7 Target cell capturing performances

In order to evaluate the target cell capturing performances by aptamers immobilized on surfaces via spacers of different lengths, target cell capturing

experiments were carried out under two different general conditions: 1) performances on flat surfaces under static conditions and 2) performances inside of microfluidic channels under flow conditions. A tumour cell lines from human acute lymphoblastic leukemia, CCRF-CEM cells were used as model cells.

To evaluate aptamer-spacer cell capturing performance on flat surfaces, seven flat surfaces of interest – unmodified PDMS surface, PAMAM dendrimer modified PDMS surface, PAMAM dendrimer modified PDMS surface further modified with either Aptamer-T2, Aptamer-T5, Aptamer-T10, Aptamer-T20 or Random-T10 were separately incubated with CCRF-CEM cells (1×10^6 cells/mL) in PBS buffer (pH 7.4) at 37 ° C for 40 min to allow cell sediment [35]. Subsequently, the flat surfaces were carefully washed by PBS buffer (pH 7.4) for 20 min. Finally, the captured CCRF-CEM cells on the surfaces were manually counted by Image J Version 1.49 (NIH, Bethesda, MD).

In order to investigate the effects of different spacer length of aptamer-spacers on cell capturing performances inside of microfluidic channels, experiments similar to those carried out on flat surfaces were performed in PDMS and SU-8 heterogeneous microfluidic channels. Briefly, CCRF-CEM cells in PBS buffer (pH 7.4) (1×10^6 cells/mL) were introduced into microfluidic channels of interest (i.e. unmodified microchannel, PAMAM modified microchannel, PAMAM modified microchannel further modified with either Aptamer-T2, Aptamer-T5, Aptamer-T10, Aptamer-T20 or Random-T10) for 40 min at a flow rate of 0.05 mL/h using a syringe pump

(Harvard Apparatus, Holliston, MA) [36]. This was followed by a washing step using PBS buffer (pH 7.4) for 20 min at 0.05 mL/h. Finally, the captured CCRF-CEM cells in microfluidic channels were manually counted by Image J Version 1.49.

5.4 Results and discussion

5.4.1 Water contact angle characterization

Water contact angles of PDMS surfaces with various modifications are demonstrated in Figure 5-2. The water contact angle of unmodified PDMS surface (Surface PDMS) was $110.6^{\circ} \pm 0.6^{\circ}$, which is consistent with the values reported previously [37, 38]. As expected, the water contact angle of PDMS surface decreased dramatically to $32.2^{\circ} \pm 0.4^{\circ}$ after the engraftment of PAMAM dendrimers (Surface PAMAM), suggesting that the PAMAM dendrimers with hydrophilic carboxyl groups have been successfully immobilized onto the PDMS surface. Furthermore, after the conjugation of aptamers, the water contact angles of the surfaces showed slight increases, exhibiting water contact angles of $45.9^{\circ} \pm 0.8^{\circ}$, $48.8^{\circ} \pm 0.9^{\circ}$, $51.9^{\circ} \pm 1.3^{\circ}$ and $61.4^{\circ} \pm 2.8^{\circ}$ for Aptamer-T2, Aptamer-T5, Aptamer-T10 and Aptamer-T20 modified surfaces, respectively. It is worthwhile to note that when different aptamers with various lengths of thymine chain spacers were used to modify the PAMAM dendrimer engrafted surfaces, longer spacers of the aptamers resulted in higher water contact angles. This is likely because by using same concentration, same

numbers of aptamers will be conjugated onto PDMS surfaces, and the aptamers with longer spacers used in the surface modification reactions tended to cover larger area on surfaces due to the extra bases, resulting in slightly increased water contact angle increased [39, 40]. Finally, the water contact angle of Random-T10 modified surface was $51.8^{\circ} \pm 1.3^{\circ}$, which has no significant difference with the water contact angle of Aptamer-T10 modified surface ($p > 0.05$, student *t*-test), suggesting that surfaces with similar hydrophilicity were obtained. It also should be mentioned that the water contact angles between any two surfaces among Aptamer-T2, Aptamer-T5, Aptamer-T10 and Aptamer-T20 modified surfaces were significantly different ($p < 0.05$, student *t*-test). The results of water contact angle characterizations of the surfaces not only showing the hydrophilicity of each modified surface but also greatly suggesting the success of surface modifications in each step on the surfaces.

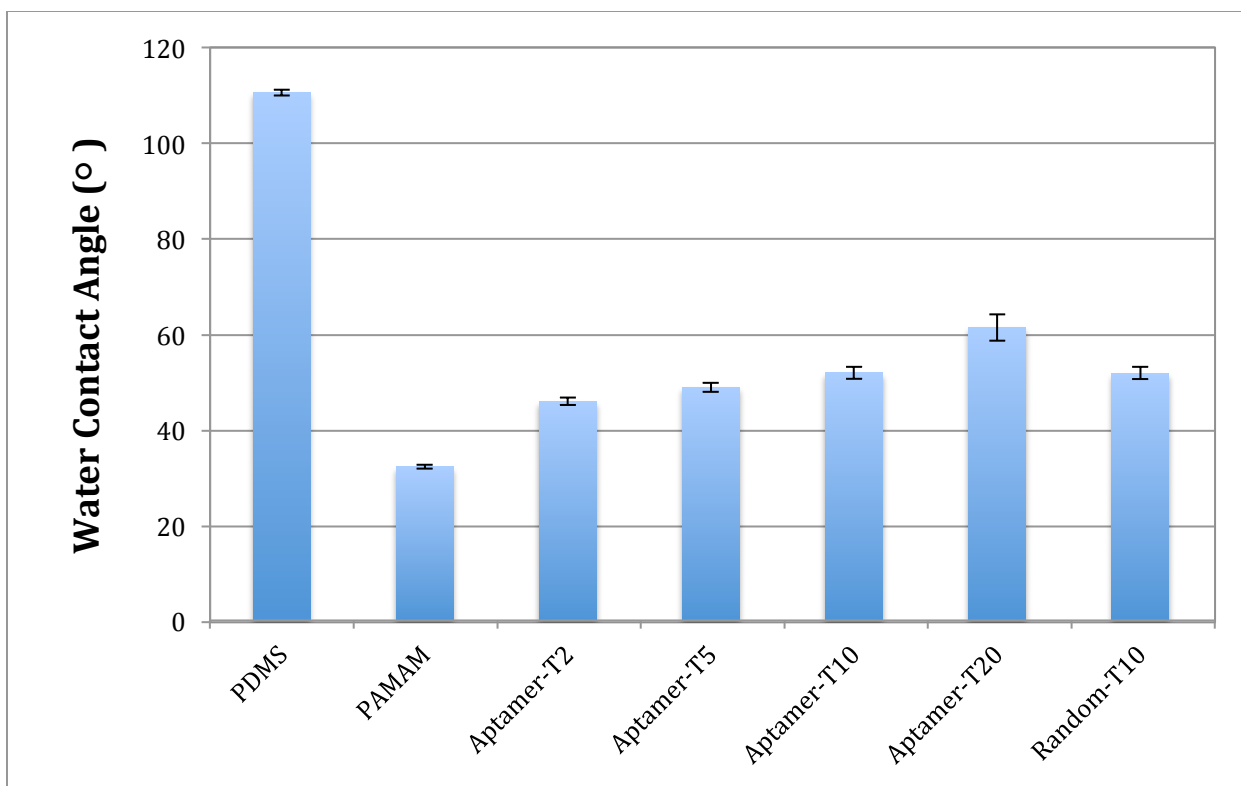


Figure 5-2. Water contact angles of surfaces with different modifications. Error bars are standard deviations of the measurements (n=4).

5.4.2 Characterization of DNA secondary structure

In order to investigate the secondary structure change of the aptamers with different length of spacers, software RNAstructure (Version 5.2) was used to simulate the secondary structures of the single strand DNA sequences. Secondary structures of Aptamer-T0 (no spacer), Aptamer-T2, Aptamer-T5, Aptamer-T10,

Aptamer-T20 and Random-T10 were simulated in fold as DNA sequence mode, and the results are shown in Figure 5-3.

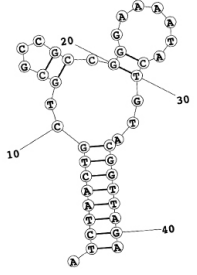
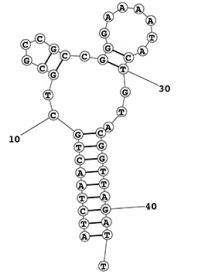
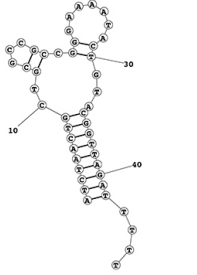
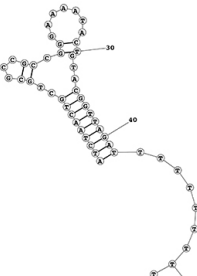
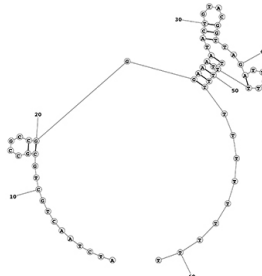
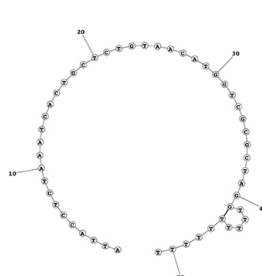
Groups	Aptamer-T0	Aptamer-T2	Aptamer-T5
Secondary structure	 (a)	 (b)	 (c)
Free Energy	- 2.5	- 3.1	- 3.1
Groups	Aptamer-T10	Aptamer-T20	Random-T10
Secondary structure	 (d)	 (e)	 (f)
Free Energy	- 3.1	- 3.8	- 1.0

Figure 5-3. Secondary structure and corresponding free energy (KJ/mol) of single strand DNA sequences simulated by RNAstructure software. Secondary structure of (a) Aptamer-T0 (no spacer), (b) Aptamer-T2, (c) Aptamer-T5, (d) Aptamer-T10, (e) Aptamer-T20 and (f) Random-T10.

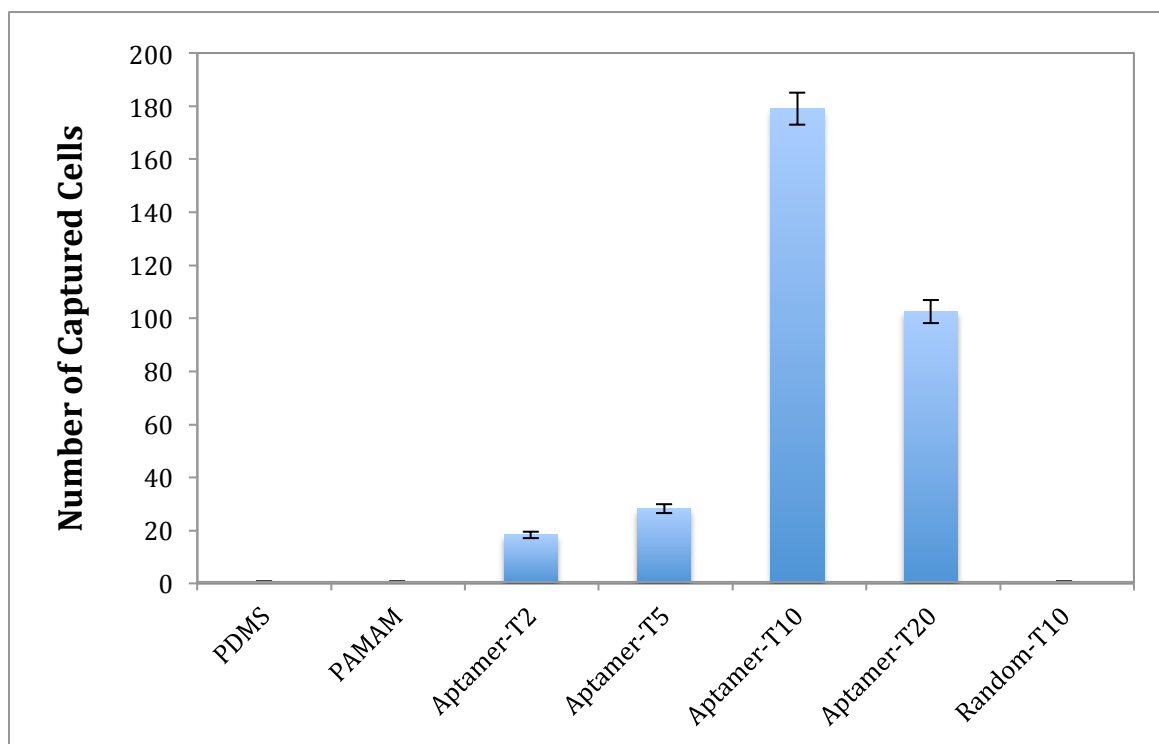
As shown in Figure 5-3, the secondary structure of sgc8 aptamer without spacer (i.e. Aptamer-T0) contained a complex stem-loop structure with two hairpin structures on one big loop (Figure 5-3(a)). Structure of the big loop started from the ninth base and ended at the thirty fourth base of the sequence, while structures of the two hairpins were from twelfth to eighteenth base and twentieth to thirtieth base, respectively. As shown in Figure 5-3(b), (c) and (d), the secondary structures of Aptamer-T2, Aptamer-T5 and Aptamer-T10 possessed the similar structures with Aptamer-T0. However, the secondary structure of Aptamer-T20 changed significantly (Figure 5-3(e)). It contained a complex stem-loop structure with one big hairpin structure, two small hairpin structures and one small loop. Structure of the big hairpin started from the twenty second base and ended at fifty second base. This big hairpin structure contained one small hairpin and one loop structure, which were from twenty eighth to thirty seventh base, and from forty first to forty sixth base, respectively. The other small hairpin structure started from thirteenth base and ended at twentieth base. As a control group, Random-T10 had a completely different structure with others, which composed of one big loop and one small loop.

5.4.3 Cell capture performances of the flat surfaces and microchannels modified with different surface modifications

In order to investigate the effects of different lengths of spacers in Aptamer-spacers on capture performance of target cells, CCRF-CEM cells were used as model cells in cell capture studies. As expected, unmodified PDMS surface and PAMAM dendrimer modified surface had almost no cell captured on the surfaces (as shown in Figure 5-4(a)). More specifically, for unmodified PDMS surface, although several cells were non-specifically adhered on the surface at the beginning because of its hydrophobic surface property, majority of the cells were easily removed after the washing step. PAMAM dendrimers provided a hydrophilic surface (see Figure 5-2), which made the surface more resistant to non-specific cell adhesion. As a result, there was no cell adherent to the PAMAM dendrimer modified surface. As shown in Figure 5-4(a), after further modifications with aptamer-spacers, surfaces with specific capturing ligands captured the target cells and the numbers of captured cells were different between the surfaces modified by aptamers with different lengths of spacers. It should be note that the surface modified by Aptamer-T2 captured the fewest target cells (17.8 ± 1.3 cells) and this number for Aptamer-T5 modified surface increased to 27.8 ± 1.7 ($p < 0.05$, student *t*-test). The number of captured cells further increased significantly to 179.0 ± 6.1 when using Aptamer-T10 as capturing ligand on the surface. However, when the surface was modified by Aptamer-T20, aptamer with even longer spacer attached, the number of captured cells decreased to 102.3 ± 4.3 , a significant decrease from capturing performance by Aptamer-T10 surface ($p < 0.05$, student *t*-test). From the results, we can see that the number of captured cells kept increasing with the length of the spacer until Aptamer-T10, and subsequently decreased when using 20 thymine bases as spacer for the aptamer. When the spacer

of an aptamer is too short (i.e. T2 and T5 in this study), the spacer could not provide sufficient space for the aptamer, which limiting its ability to capture target cells. The previous results of aptamer structure simulation (see Figure 5-3) have shown that the secondary structure of Aptamer-T20 changed to another structure that significantly different from secondary structure of Aptamer-T0, Aptamer-T2, Aptamer-T5 and Aptamer-T10. When combine this result with the result of cell capture study, it should be noted that when the spacer of an aptamer is too long (i.e. T20 in this study), although the aptamer has larger space to form its secondary structure, the long spacer may lead to unexpected base pairings between the bases in aptamer and the bases in spacer, resulting in different secondary structure formation of the aptamer and loss of its capture ability [41, 42]. Fortunately, 10-thymine bases spacer has the appropriate length for sgc8 aptamer to form its secondary structure and finally capture its target. As a control group, the surface modified by Random-T10 had no cell been captured on the surface (as shown in Figure 5-4(a)).

(a)



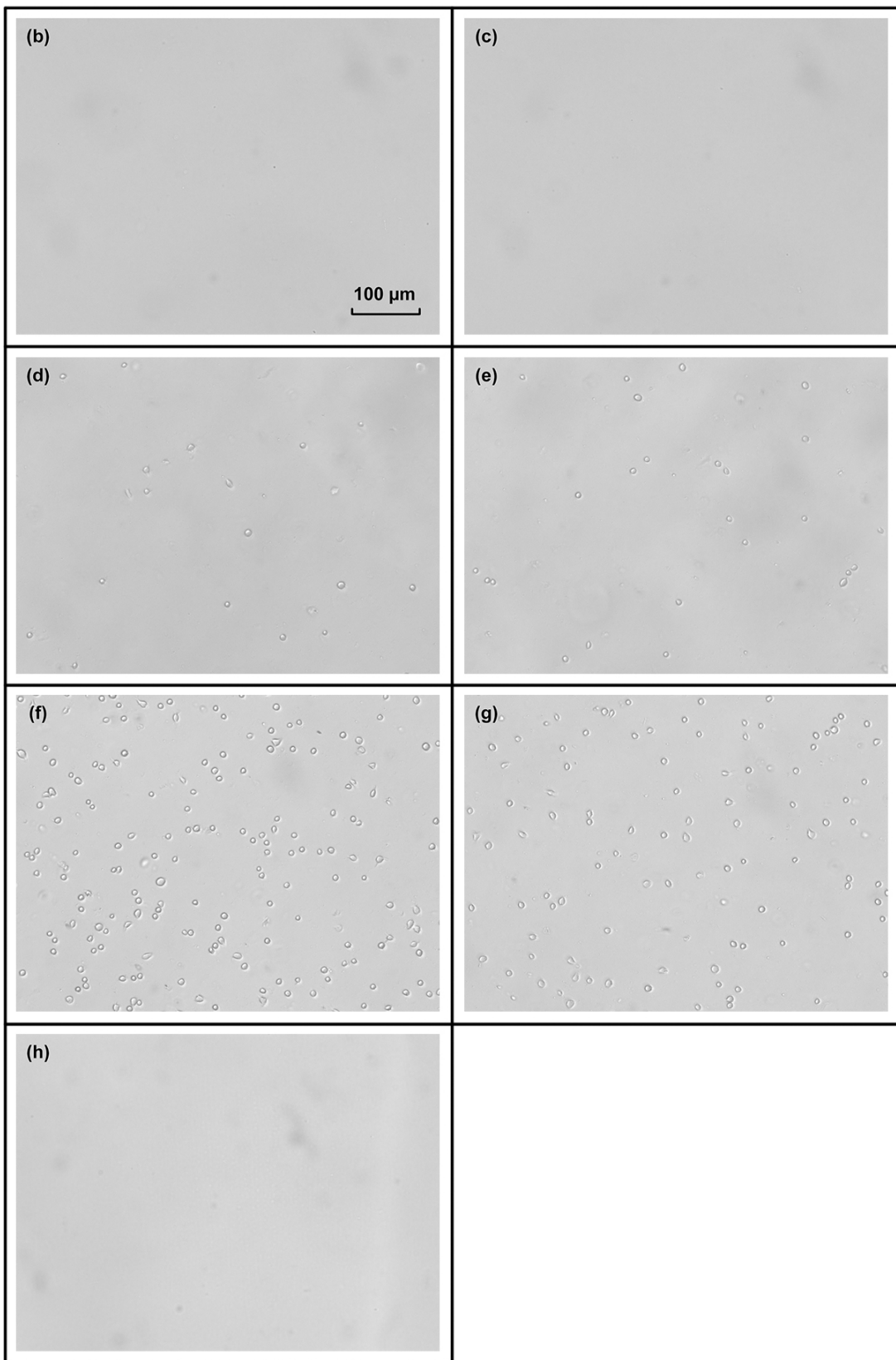


Figure 5-4. Target cell capture performances of PDMS surfaces with different surface modifications. (a) bar plot demonstrating average numbers of captured cells in different and random views on different surfaces. Error bars are standard deviations of the measurements (n=4). (b-h) micrographs of target cells captured on surfaces with different surface modifications: (b) original PDMS surface, (c) PDMS surface modified with PAMAM dendrimer, (d) PAMAM engrafted PDMS surface further modified by Aptamer-T2, (e) PAMAM engrafted PDMS surface further modified by Aptamer-T5, (f) PAMAM engrafted PDMS surface further modified by Aptamer-T10, (g) PAMAM engrafted PDMS surface further modified by Aptamer-T20 and (h) PAMAM engrafted PDMS surface further modified by Random-T10.

In order to further demonstrate the specificity between sgc8 aptamer and CCRF-CEM cell (i.e. target cell), Ramos cells were used as control cells for CCRF-CEM cells. As shown in Figure 5-5, there was no Ramos cell captured on either Aptamer-T2, Aptamer-T5, Aptamer-T10 or Aptamer-T20 modified surfaces. The target cell capture performance of Aptamer-T2, Aptamer-T5, Aptamer-T10 and Aptamer-T20 modified surfaces, combined with surface performance of Random-T10 control group and Ramos cell control group greatly suggesting the specificity between sgc8 aptamers and CCRF-CEM target cells.

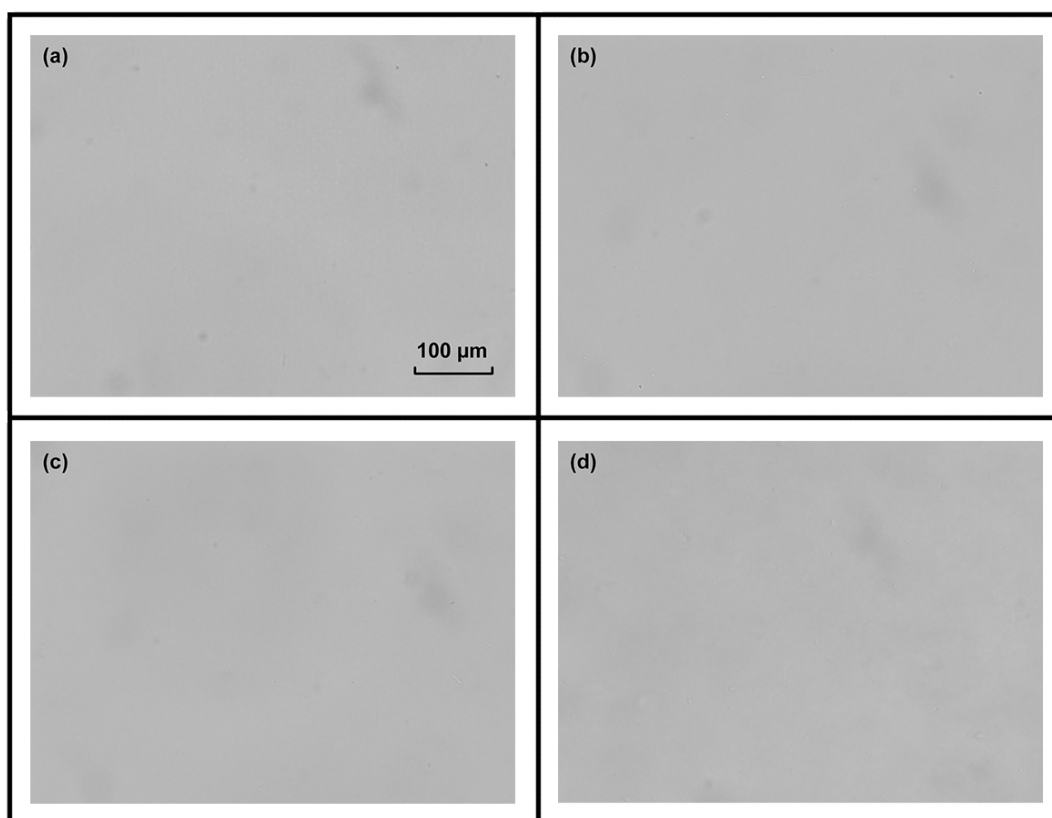
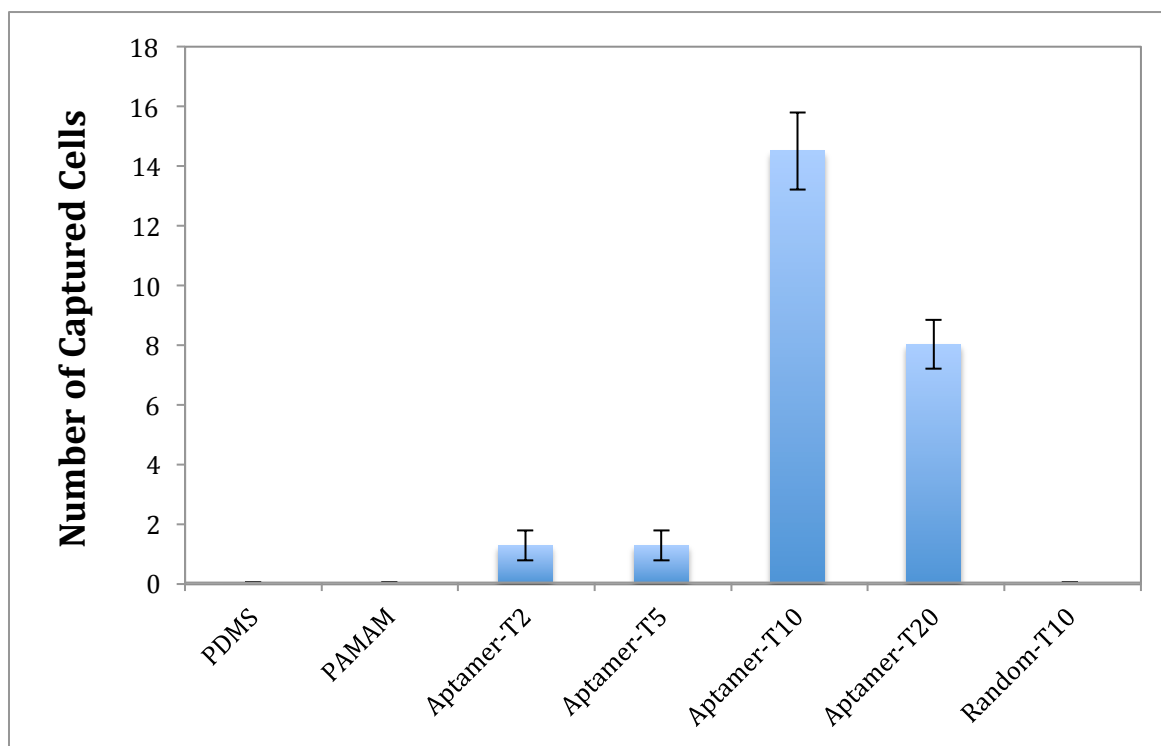


Figure 5-5. Control cell capture performances of PDMS surfaces with different surface modifications. (a-d) micrographs of surfaces after the treatment of control cells: (a) PAMAM engrafted PDMS surface further modified by Aptamer-T2, (b) PAMAM engrafted PDMS surface further modified by Aptamer-T5, (c) PAMAM engrafted PDMS surface further modified by Aptamer-T10, (d) PAMAM engrafted PDMS surface further modified by Aptamer-T20.

Similarly, cell capturing performances of microfluidic channels with different modifications were also investigated, and the results were consistent with the study on flat surfaces. As shown in Figure 5-6, Aptamer-T10 modified surface captured

significantly more target cells than either Aptamer-T2, Aptamer-T5 or Aptamer-T20 modified surfaces, suggesting that ten thymine bases of the spacers was the best length for sgc8 aptamer on target cell capture. The number of captured cells on Aptamer-T20 modified surface was fewer than Aptamer-T10 modified surface but significantly more than that of Aptamer-T2 and Aptamer-T5 modified surfaces, suggesting that twenty thymine bases spacers provided more space for sgc8 aptamers to form their secondary structures, however, long spacers may trigger unexpected base pairings between aptamers and spacers at the same time. It should be mentioned that the numbers of captured cells in Aptamer-T2 and Aptamer-T5 modified microfluidic channels were not significantly different ($p>0.05$, student *t*-test). It also should be noted that under flow condition in microchannels, the numbers of captured cells in microchannels were smaller than that on flat surfaces because of the shear force in microchannels [43, 44].

(a)



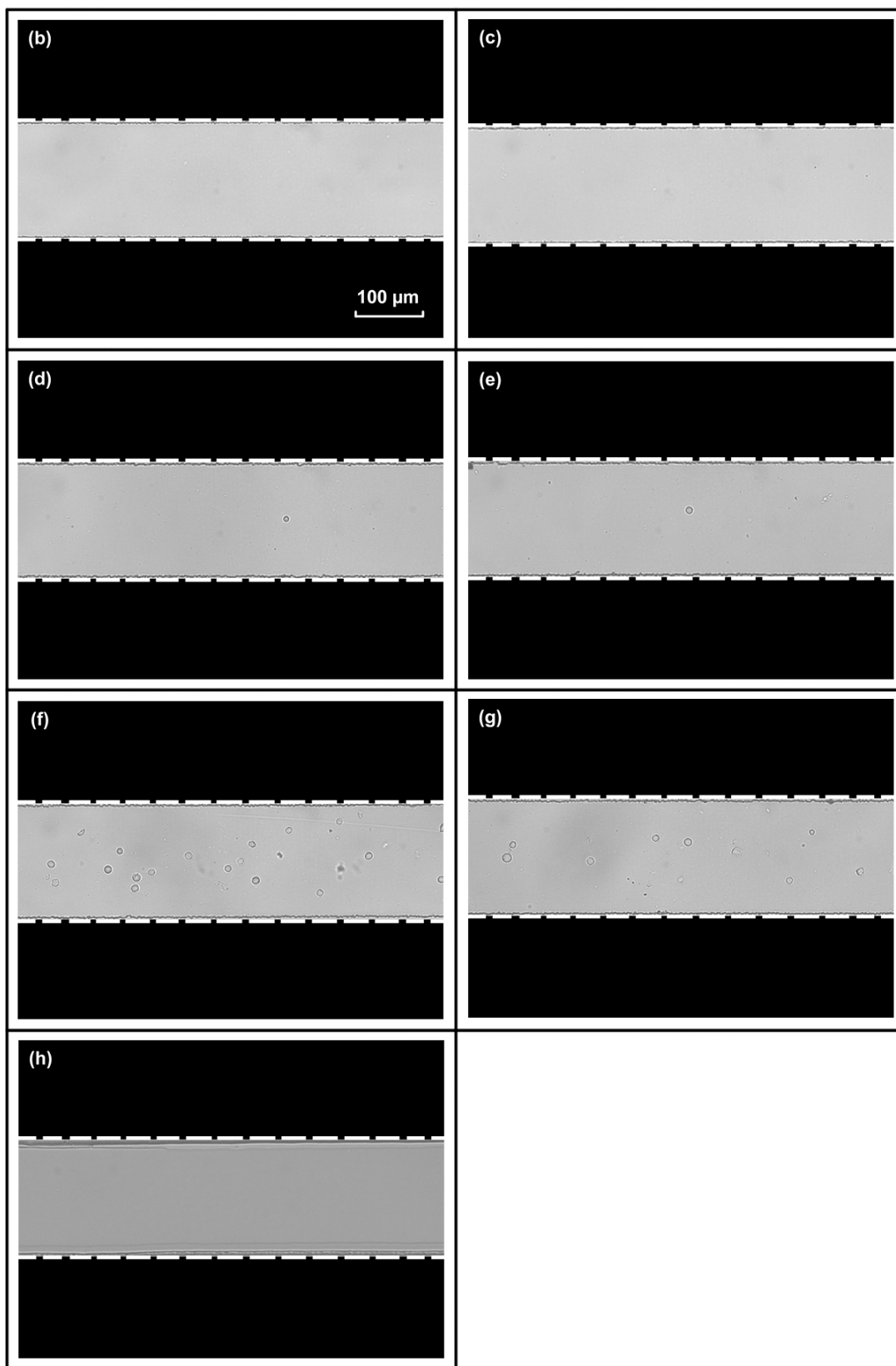


Figure 5-6. Target cell capture performances of microfluidic channels with different surface modifications. (a) bar plot demonstrating average numbers of captured cells in different and random views on different surfaces in microchannels. Error bars are standard deviations of the measurements (n=4). (b-h) micrographs of target cells captured on different surfaces in microchannels: (b) original PDMS surface, (c) PDMS surface modified with PAMAM dendrimer, (d) PAMAM engrafted PDMS surface further modified by Aptamer-T2, (e) PAMAM engrafted PDMS surface further modified by Aptamer-T5, (f) PAMAM engrafted PDMS surface further modified by Aptamer-T10, (g) PAMAM engrafted PDMS surface further modified by Aptamer-T20 and (h) PAMAM engrafted PDMS surface further modified by Random-T10.

Given the results demonstrated above, it is obvious that in designing a spacer of an aptamer for high sensitivity detection applications, one should consider not only the type of the spacer to achieve high affinity of the aptamer but also the length of the spacer to achieve high probability of secondary structure of the aptamer. Spacers shorter than ten thymine bases will result in lack of space for aptamers that limiting their ability to capture target cells, while longer spacers may lead to unexpected base pairings between the spacers and aptamers, both of which will result in lower probability of secondary structure formation of the aptamers and loss of their functions for target capturing. To the best of our knowledge, this is the first time

studying the influences made by a wide range of spacer length to aptamers on their target capture performance in microfluidic systems.

5.5 Conclusions

In this study, we designed different surfaces modified by aptamers with various lengths of spacers to investigate the effects made by spacer length on cell capture performance. Using CCRF-CEM cells as model cells for CTCs on both flat surfaces and surfaces in microchannels, we show that ten thymine bases spacer has the best length as a spacer for sgc8 aptamer for CCRF-CEM cell capture under the experimental conditions used in this study. Spacers shorter than ten thymine bases will result in lack of space for aptamers that limiting their ability to capture target cells. However, when the spacers of the aptamers are in an excessive length, the secondary structure formation of partial aptamers will change and lose their ability to capture the target cells. We also present a surface modification strategy by using highly branched polymer, PAMAM dendrimers that can not only provide large loading capacity for aptamers by their multi-functional sites but also decrease background signals by their hydrophilic surface property, which is significantly important for preparing lab-on-a-chip microfluidic systems for high sensitivity biosensing and detection applications.

5.6 References

- [1] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, Global cancer statistics, *CA: a cancer journal for clinicians*, 61 (2011) 69-90.
- [2] I.J. Fidler, The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited, *Nature Reviews Cancer*, 3 (2003) 453-458.
- [3] M.S. Wicha, D.F. Hayes, Circulating tumor cells: not all detected cells are bad and not all bad cells are detected, *Journal of Clinical Oncology*, 29 (2011) 1508-1511.
- [4] P.S. Steeg, Tumor metastasis: mechanistic insights and clinical challenges, *Nature medicine*, 12 (2006) 895-904.
- [5] G.P. Gupta, J. Massagué, Cancer metastasis: building a framework, *Cell*, 127 (2006) 679-695.
- [6] T.N. Seyfried, L.C. Huysentruyt, On the origin of cancer metastasis, *Critical reviews in oncogenesis*, 18 (2013) 43.
- [7] V. Souza e Silva, M.F. Fanelli, F.A. Soares, T.C. Felismino, M. Eliza Cavicchioli Buim, V.A. Silva, B. Maria Malagoli Rocha, L.M.M. Ocea, N.B. Mingues, J.L.G. Junior, Evaluation of circulating tumor cells (CTCs) as a potential biomarker in patients with colorectal cancer (CRC): A prospective analysis, *American Society of Clinical Oncology*, 2014.
- [8] A. Alva, T. Friedlander, M. Clark, T. Huebner, S. Daignault, M. Hussain, C. Lee, K. Hafez, B. Hollenbeck, A. Weizer, Circulating tumor cells as potential biomarkers in bladder cancer, *The Journal of urology*, 194 (2015) 790-798.
- [9] D.C. Danila, M. Fleisher, H.I. Scher, Circulating tumor cells as biomarkers in prostate cancer, *Clinical Cancer Research*, 17 (2011) 3903-3912.
- [10] K. Pantel, C. Alix-Panabières, Detection methods of circulating tumor cells, *Journal of thoracic disease*, 4 (2012) 446.
- [11] W.J. Allard, J. Matera, M.C. Miller, M. Repollet, M.C. Connelly, C. Rao, A.G. Tibbe, J.W. Uhr, L.W. Terstappen, Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases, *Clinical Cancer Research*, 10 (2004) 6897-6904.
- [12] E. Racila, D. Euhus, A.J. Weiss, C. Rao, J. McConnell, L.W. Terstappen, J.W. Uhr, Detection and characterization of carcinoma cells in the blood, *Proceedings of the National Academy of Sciences*, 95 (1998) 4589-4594.
- [13] P. Pinzani, B. Salvadori, L. Simi, S. Bianchi, V. Distanti, L. Cataliotti, M. Pazzagli, C. Orlando, Isolation by size of epithelial tumor cells in peripheral blood of patients with breast cancer: correlation with real-time reverse transcriptase-polymerase chain reaction results and feasibility of molecular analysis by laser microdissection, *Human pathology*, 37 (2006) 711-718.
- [14] W. Liu, X. Zhou, D. Xing, Rapid and reliable microRNA detection by stacking hybridization on electrochemiluminescent chip system, *Biosensors and Bioelectronics*, 58 (2014) 388-394.

- [15] J. Horak, C. Dincer, H. Bakirci, G. Urban, Sensitive, rapid and quantitative detection of substance P in serum samples using an integrated microfluidic immunochip, *Biosensors and Bioelectronics*, 58 (2014) 186-192.
- [16] V.J. Ruigrok, M. Levisson, M.H. Eppink, H. Smidt, J. Van Der Oost, Alternative affinity tools: more attractive than antibodies?, *Biochemical Journal*, 436 (2011) 1-13.
- [17] A.B. Iliuk, L. Hu, W.A. Tao, Aptamer in bioanalytical applications, *Analytical chemistry*, 83 (2011) 4440-4452.
- [18] Y. Xu, J.A. Phillips, J. Yan, Q. Li, Z.H. Fan, W. Tan, Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells, *Analytical chemistry*, 81 (2009) 7436-7442.
- [19] W. Sheng, T. Chen, R. Kamath, X. Xiong, W. Tan, Z.H. Fan, Aptamer-enabled efficient isolation of cancer cells from whole blood using a microfluidic device, *Analytical chemistry*, 84 (2012) 4199-4206.
- [20] L. Cao, L. Cheng, Z. Zhang, Y. Wang, X. Zhang, H. Chen, B. Liu, S. Zhang, J. Kong, Visual and high-throughput detection of cancer cells using a graphene oxide-based FRET aptasensing microfluidic chip, *Lab on a Chip*, 12 (2012) 4864-4869.
- [21] R. Kong, Z. Chen, M. Ye, X. Zhang, W. Tan, Cell-SELEX-based aptamer-conjugated nanomaterials for enhanced targeting of cancer cells, *Science China Chemistry*, 54 (2011) 1218.
- [22] B. Waybrant, T.R. Pearce, E. Kokkoli, Effect of polyethylene glycol, alkyl, and oligonucleotide spacers on the binding, secondary structure, and self-assembly of fractalkine binding FKN-S2 aptamer-amphiphiles, *Langmuir*, 30 (2014) 7465-7474.
- [23] E. Southern, K. Mir, M. Shchepinov, Molecular interactions on microarrays, *Nature genetics*, 21 (1999) 5-9.
- [24] Y.-H. Lao, K. Peck, L.-C. Chen, Enhancement of aptamer microarray sensitivity through spacer optimization and avidity effect, *Analytical chemistry*, 81 (2009) 1747-1754.
- [25] L. Zhou, L.-J. Ou, X. Chu, G.-L. Shen, R.-Q. Yu, Aptamer-based rolling circle amplification: a platform for electrochemical detection of protein, *Analytical chemistry*, 79 (2007) 7492-7500.
- [26] S.J. Lee, B.-S. Youn, J.W. Park, J.H. Niazi, Y.S. Kim, M.B. Gu, ssDNA aptamer-based surface plasmon resonance biosensor for the detection of retinol binding protein 4 for the early diagnosis of type 2 diabetes, *Analytical chemistry*, 80 (2008) 2867-2873.
- [27] P.Y. Yeh, Z. Zhang, M. Lin, X. Cao, Nonfouling hydrophilic poly (ethylene glycol) engraftment strategy for PDMS/SU-8 heterogeneous microfluidic devices, *Langmuir*, 28 (2012) 16227-16236.
- [28] J.K. Herr, J.E. Smith, C.D. Medley, D. Shangguan, W. Tan, Aptamer-conjugated nanoparticles for selective collection and detection of cancer cells, *Analytical Chemistry*, 78 (2006) 2918-2924.
- [29] D. Shangguan, Y. Li, Z. Tang, Z.C. Cao, H.W. Chen, P. Mallikaratchy, K. Sefah, C.J. Yang, W. Tan, Aptamers evolved from live cells as effective molecular probes for cancer study, *Proceedings of the National Academy of Sciences*, 103 (2006) 11838-11843.

- [30] E. Ouellet, C.W.T. Yang, T. Lin, L.L. Yang, E.T. Lagally, Novel carboxyl-amine bonding methods for poly (dimethylsiloxane)-based devices, *Langmuir*, 26 (2010) 11609-11614.
- [31] Z. Zhang, P. Zhao, G. Xiao, The fabrication of polymer microfluidic devices using a solid-to-solid interfacial polyaddition, *Polymer*, 50 (2009) 5358-5361.
- [32] Y. Qin, P. Yeh, X. Hao, X. Cao, Developing an ultra non-fouling SU-8 and PDMS hybrid microfluidic device by poly (amidoamine) engraftment, *Colloids and Surfaces B: Biointerfaces*, 127 (2015) 247-255.
- [33] D.H. Mathews, Using the RNAstructure Software Package to predict conserved RNA structures, *Current protocols in bioinformatics*, (2014) 12.14. 11-12.14. 22.
- [34] S. Bellaousov, J.S. Reuter, M.G. Seetin, D.H. Mathews, RNAstructure: Web servers for RNA secondary structure prediction and analysis, *Nucleic acids research*, 41 (2013) W471-W474.
- [35] D. Yongabi, M. Khorshid, P. Losada-Pérez, K. Eersels, O. Deschaume, J. D'Haen, C. Bartic, J. Hooyberghs, R. Thoelen, M. Wübbenhorst, Cell detection by surface imprinted polymers SIPs: A study to unravel the recognition mechanisms, *Sensors and Actuators B: Chemical*, 255 (2018) 907-917.
- [36] F. Walther, P. Davydovskaya, S. Zürcher, M. Kaiser, H. Herberg, A.M. Gigler, R.W. Stark, Stability of the hydrophilic behavior of oxygen plasma activated SU-8, *Journal of Micromechanics and Microengineering*, 17 (2007) 524.
- [37] S. Prabakaran, S. Jayaraman, D.H. Minh, S.K. Sinha, The role of functional end groups of perfluoropolyether (Z-dol and Z-03) lubricants in augmenting the tribology of SU-8 composites, *Tribology Letters*, 56 (2014) 423-434.
- [38] G. Li, L. Shen, Y. Luo, S. Zhang, The effect of silver-PAMAM dendrimer nanocomposites on the performance of PVDF membranes, *Desalination*, 338 (2014) 115-120.
- [39] H.O. Ham, Z. Liu, K. Lau, H. Lee, P.B. Messersmith, Facile DNA immobilization on surfaces through a catecholamine polymer, *Angewandte Chemie*, 123 (2011) 758-762.
- [40] S. Taylor, S. Smith, B. Windle, A. Guiseppi - Elie, Impact of surface chemistry and blocking strategies on DNA microarrays, *Nucleic Acids Research*, 31 (2003) e87-e87.
- [41] G. Zhu, M. Lübbecke, J.G. Walter, F. Stahl, T. Scheper, Characterization of Optimal Aptamer - Microarray Binding Chemistry and Spacer Design, *Chemical Engineering & Technology*, 34 (2011) 2022-2028.
- [42] M. Witt, J.-G. Walter, F. Stahl, Aptamer microarrays—Current status and future prospects, *Microarrays*, 4 (2015) 115-132.
- [43] T. Thorsen, R.W. Roberts, F.H. Arnold, S.R. Quake, Dynamic pattern formation in a vesicle-generating microfluidic device, *Physical review letters*, 86 (2001) 4163.
- [44] J. Xu, S. Li, J. Tan, Y. Wang, G. Luo, Preparation of highly monodisperse droplet in a T - junction microfluidic device, *AIChE journal*, 52 (2006) 3005-3010.

Chapter 6 General discussion and Conclusions

6.1 Discussion

It is clear that the bonding strength between surfaces in a microfluidic device indicates the quality of the device, which is significantly important for the stability and capability of the microfluidic system. Time of oxygen plasma treatment and concentration of APTMS could affect the bonding strength between PDMS and SU-8 surfaces and were set as variables in this project. The results showed that PDMS surfaces treated with oxygen plasma for 10 s and subsequently modified with 0.05 wt.% APTMS provided the strongest bonding strength with SU-8 surface. This is because long time treatment of oxygen plasma would damage the backbone structure of PDMS, resulting in efficacy loss of the PDMS surface [1]. In terms of SU-8 surface amination, higher concentration of EDA solution resulted in more amino functional groups on the SU-8 surface. However, the concentration of EDA solution should be optimized at an appropriate value based on the size of the microchannel. This is because microchannel in small size might be blocked if the concentration of EDA solution was relatively high. In the characterization result of SU-8 surface amination, it also should be mentioned that the fluorescence intensity of background was higher than that of PDMS surface. This is because SU-8 surface is slightly auto-fluorescent at several excitation wavelengths [2, 3]. For the non-fouling performance of hybrid microchannels, the results showed that higher concentration of PAMAM dendrimers resulted in fewer non-specific adsorptions. When the concentrations of PAMAM dendrimers were higher than 4.0 μM , there was no

significant difference between the numbers of non-specifically adsorbed particles or cells on the surfaces in microfluidic channels.

According to the results of surface morphology characterization, PDMS and SU-8 surfaces are smooth surfaces before modification. The roughness of both surfaces slightly increased after surface amination by APTMS treatment to PDMS surface and EDA treatment to SU-8 surface. After PAMAM modification, roughness of both PDMS and SU-8 surfaces increased significantly. This surface morphology change is likely because of the three dimensional molecular packing of PAMAM dendrimers on the surfaces [4]. It also should be mentioned that as the concentrations of PAMAM dendrimers increased, the roughness of both PDMS and SU-8 surfaces increased as well. This is because of the increased PAMAM dendrimer aggregation on the resulting surfaces [5, 6]. When evaluating the non-fouling performance of PDMS surfaces to dead and live CTCs, the results showed that the largest number of adsorbed cells happened between unmodified PDMS surfaces and dead CTCs. The reason is hydrophobic interaction. Briefly, hydrophobic molecules are more likely to aggregate together rather than distributing themselves. This is because the combination between hydrophobic molecules allows them to have minimal contact with water [7]. Since the surfaces of live cells are more hydrophilic than dead cells, dead cells are more like to be attached on the unmodified PDMS surface, which is highly hydrophobic [8]. This principle can also explain the non-fouling performance of PAMAM dendrimer modified surfaces. The hydrophilic surface of PAMAM dendrimers successfully avoided the contact between non-specific particles or cells

with PDMS surface. Moreover, PAMAM dendrimers are highly branched macromolecules with high extension and chain flexibility, which can protect the surfaces from being fouled through steric interactions [9].

After modifications of aptamers with different lengths of spacers, the water contact angles demonstrated that longer spacer resulted in higher contact angle. This is likely because aptamers tethered to the surfaces using longer spacers would most likely result in larger surface coverage by the DNA aptamers that are known to render surfaces more hydrophobic when coated on surfaces [10, 11]. The numbers of captured CTCs by aptamers with different lengths of spacers showed an increase trend until the length of spacer reached 10 thymine bases, and subsequently decreased when using 20 thymine bases as spacer. This phenomenon can be explained by the simulation results of aptamer secondary structures. Secondary structure of an aptamer is formed by the complementary pairings between the bases in the aptamer. When linking longer spacer to an aptamer, the bases in spacer may trigger irregular folding of the aptamer and result in loss of its capture ability [12, 13]. It should be noted that the numbers of captured cells in microchannels were generally smaller than that on flat surfaces, this is because when compared with cells on surfaces, the captures of cells in microchannels need to overcome the influence made by shear force in the microfluidic channels [14, 15].

6.2 Conclusion

In this project, the main objective is to provide a PDMS/SU-8 hybrid microfluidic system that could sensitively detect CTCs with excellent non-fouling features. Here is the strategies and conclusions to make the system based on the study of this project: (1) Bonding between PDMS and SU-8 surfaces was based on the reactions between the amino functional groups on APTMS modified PDMS surface and the epoxy functional groups on SU-8 surface. In the process of PDMS surface amination, PDMS surface treated with oxygen plasma for 10 s and subsequently modified with 0.05 wt.% APTMS forms the strongest interface with SU-8 surface. (2) In the process of SU-8 surface amination, 20 wt.% of EDA in anhydrous ethanol was an appropriate concentration for this study. Although 30 wt.% of EDA in anhydrous ethanol provide more amino functional groups on SU-8 surface, one should consider the concentration of EDA based on the size of the microchannels. This is because microchannel in small size might be blocked if the concentration of EDA solution was relatively high. (3) As shown in Chapter 3, by introducing PAMAM dendrimers in PDMS/SU-8 hybrid microchannel, the non-fouling performance of the microchannel had been significantly improved. (4) 4.0 μM of PAMAM dendrimer was the best concentration in this study. Further increase of the concentration did not result in better non-fouling performance with significant difference. (5) As shown in Chapter 4, by using both live and dead CCRF-CEM cells as model cells for CTCs, the results showed that for live cell, electric charges of a surface is the most important factor affecting the non-fouling properties of the surface in microfluidic

systems while for dead cells, surface hydrophilicity is the most important factor affecting surface non-fouling features. However, surface roughness does not seem to be as important for both live and dead cells under the experimental conditions used in this study. (6) The interactions between microfluidic channel surfaces and cells (i.e. live and dead) are significantly different. One should consider not only the surface properties (i.e. hydrophilicity/hydrophobicity) of the microfluidic device but also the electric charge properties of the targets as well as those of the non-targets. (7) As shown in Chapter 5, ten thymine bases spacer has the best length as a spacer for sgc8 aptamer for CCRF-CEM cell capture under the experimental conditions used in this study. (8) Spacers for sgc8 aptamer shorter than ten thymine bases will result in lack of space for aptamers to form their secondary structures in order to capture the target cells. However, when the spacers of the aptamers are in an excessive length, the secondary structure formation of partial aptamers will change and lose their ability to capture the target cells. (9) PAMAM dendrimers that can not only provide large loading capacity for aptamers by their multi-functional sites but also decrease background signals by their hydrophilic surface property, which is significantly important for preparing lab-on-a-chip microfluidic systems for high sensitivity biosensing and detection applications.

6.3 Recommendation

The following are a few recommendations for future research directions regarding to this study.

In this project, the fluid in microchannels moved in a laminar flow pattern, which limited the contact opportunities of the CTCs with the aptamers tethered to the surfaces of the microchannels. To further enhance the capability of our designed system, the microchannels can be improved with patterns at the bottom surface to generate disturbance in the microchannels. For example, Xue et al. reported a microfluidic device with herringbone grooves at the bottom of the microchannels to detect Hep3B (hepatocellular carcinoma) tumour cells [16]; Chen et al. demonstrated another microfluidic system with micropillars at the bottom of the microchannels for MCF-7 breast cancer cells detection [17]. However, these microfluidic systems do not have surface modification for the prevention of non-specific adsorptions. If we combine our strategy of surface modification with the patterned microfluidic channels, the interactions between the aptamers on surfaces in microchannel and the target CTCs will be more effective. It should be noted that when designing patterns in microfluidic channels, advance simulation should be performed by software such as COMSOL simulation (COMSOL Multiphysics). Moreover, the PDMS/SU-8 hybrid microfluidic system can be improved by combination with graphene oxide. Graphene oxide has been used in the field of detection as an FRET (fluorescence resonance energy transfer) acceptor. With the application of graphene oxide, quenching and recovery of fluorescence in microfluidic systems could make the detection significantly sensitive. By introducing graphene oxide into our microfluidic system, PAMAM dendrimer is used as material to decrease background signals while graphene oxide is applied as

material to increase limit of detection (LOD), both of which exist for the improvement of detection sensitivity in the microfluidic system.

6.4 References

- [1] S. Bhattacharya, A. Datta, J.M. Berg, S. Gangopadhyay, Studies on surface wettability of poly (dimethyl) siloxane (PDMS) and glass under oxygen-plasma treatment and correlation with bond strength, *Journal of microelectromechanical systems*, 14 (2005) 590-597.
- [2] G. Blagoi, S. Keller, A. Johansson, A. Boisen, M. Dufva, Functionalization of SU-8 photoresist surfaces with IgG proteins, *Applied Surface Science*, 255 (2008) 2896-2902.
- [3] R. Marie, S. Schmid, A. Johansson, L. Ejsing, M. Nordström, D. Häfliger, C.B. Christensen, A. Boisen, M. Dufva, Immobilisation of DNA to polymerised SU-8 photoresist, *Biosensors and Bioelectronics*, 21 (2006) 1327-1332.
- [4] G. Li, L. Shen, Y. Luo, S. Zhang, The effect of silver-PAMAM dendrimer nanocomposites on the performance of PVDF membranes, *Desalination*, 338 (2014) 115-120.
- [5] M.J. Jasmine, E. Prasad, Fractal growth of PAMAM dendrimer aggregates and its impact on the intrinsic emission properties, *The Journal of Physical Chemistry B*, 114 (2010) 7735-7742.
- [6] H.A. Essawy, H.A. Mohamed, Poly (amidoamine) dendritic structures, bearing different end groups, as adhesion promoters for urea-formaldehyde wood adhesive system, *Journal of Applied Polymer Science*, 119 (2011) 760-767.
- [7] D. Chandler, Hydrophobicity: Two faces of water, *Nature*, 417 (2002) 491-491.
- [8] M.S. Bretscher, Membrane structure: some general principles, *Science*, 181 (1973) 622-629.
- [9] P.-Y. Yeh, J.N. Kizhakkedathu, M. Chiao, A novel method to attenuate protein adsorption using combinations of polyethylene glycol (PEG) grafts and piezoelectric actuation, *Journal of Nanotechnology in Engineering and Medicine*, 1 (2010) 041010.
- [10] H.O. Ham, Z. Liu, K. Lau, H. Lee, P.B. Messersmith, Facile DNA immobilization on surfaces through a catecholamine polymer, *Angewandte Chemie*, 123 (2011) 758-762.
- [11] S. Taylor, S. Smith, B. Windle, A. Guiseppi - Elie, Impact of surface chemistry and blocking strategies on DNA microarrays, *Nucleic Acids Research*, 31 (2003) e87-e87.
- [12] G. Zhu, M. Lübbecke, J.G. Walter, F. Stahl, T. Scheper, Characterization of Optimal Aptamer - Microarray Binding Chemistry and Spacer Design, *Chemical Engineering & Technology*, 34 (2011) 2022-2028.
- [13] M. Witt, J.-G. Walter, F. Stahl, Aptamer microarrays—Current status and future prospects, *Microarrays*, 4 (2015) 115-132.
- [14] T. Thorsen, R.W. Roberts, F.H. Arnold, S.R. Quake, Dynamic pattern formation in a vesicle-generating microfluidic device, *Physical review letters*, 86 (2001) 4163.
- [15] J. Xu, S. Li, J. Tan, Y. Wang, G. Luo, Preparation of highly monodisperse droplet in a T - junction microfluidic device, *AIChE journal*, 52 (2006) 3005-3010.

- [16] P. Xue, Y. Wu, J. Guo, Y. Kang, Highly efficient capture and harvest of circulating tumor cells on a microfluidic chip integrated with herringbone and micropost arrays, *Biomedical microdevices*, 17 (2015) 39.
- [17] H. Chen, B. Cao, H. Chen, Y.-S. Lin, J. Zhang, Combination of antibody-coated, physical-based microfluidic chip with wave-shaped arrays for isolating circulating tumor cells, *Biomedical microdevices*, 19 (2017) 66.

Appendix A

Preparation of PAMAM-COOH

In a typical reaction to convert original PAMAM-NH₂ to PAMAM-COOH, 0.02 g of succinic anhydride (2×10^{-4} mol) and 0.3 g of PAMAM solution (5% solution in methanol, 1.3×10^{-7} mol) were mixed in 2 mL PBS (pH 7.4) to allow reaction to proceed for 4 h at room temperature under stirring. The reaction mixture was then dialyzed against DI water using a Slide-A-Lyzer Dialysis Cassette (10k MWCO, Thermo Scientific) under gentle agitation overnight. After dialysis, the resulting modified product (i.e. PAMAM-COOH) was kept in at 4 °C until future use.

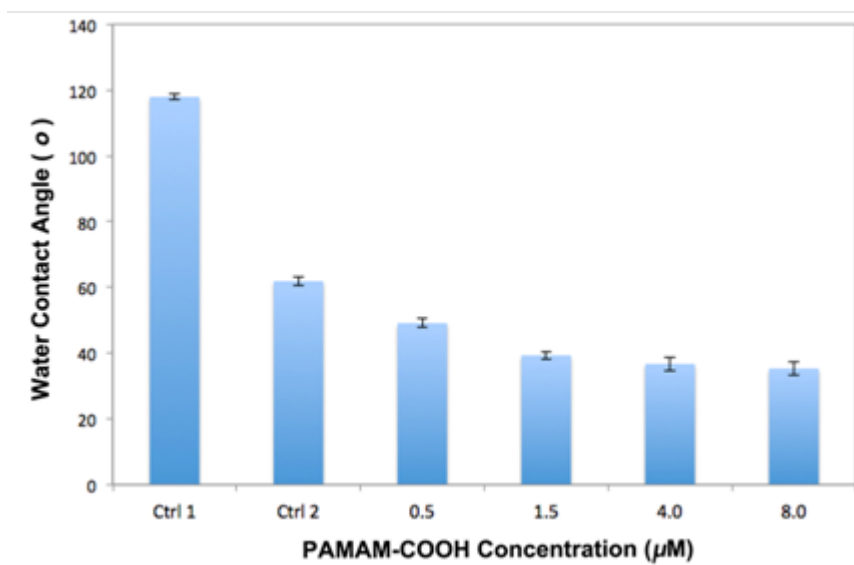


Figure S1. Water contact angle measurements of PAMAM modified PDMS surfaces using different bulk PAMAM-COOH concentrations. In the figure, Ctrl 1 = unmodified PDMS surface and Ctrl 2 = aminated PDMS surface. Standard deviation of the measurements was shown as error bars (n=6).

Washing process of microbeads

After purchase from the manufacture, the microbeads need to be washed and resuspended to remove the surfactants before use. The microbeads were washed in 5 mL of DI water via bath sonication for 5min, and then separated via centrifugation at $1200 \times g$ for 15 min, followed by another sonication for 5 min for resuspension at concentration of 1×10^8 particles/mL for further tests.

Detailed Data of adhesion studies

In the following two tables, numbers of the adhered particles and the corresponding suppression ratios are shown.

Table S1. Numbers and suppression ratios of adhered fluorescent microbeads in modified microchannels (n=4)

Samples	Unmod-ified	Con 1	Con2	Con3	Con4	Con5	Con6	Con7	Con8	Con9
Average number of adhered microbeads	25554	437	330	258	190	74	81	195	82	82
Suppression ratio (%)	/	98.29 ± 0.35	98.71 ± 0.40	98.99 ± 0.31	99.26 ± 0.24	99.64 ± 0.25	99.68 ± 0.17	99.24 ± 0.22	99.68 ± 0.17	99.68 ± 0.15

Table S2. Numbers and suppression ratios of adhered *E. coli* cells in unmodified and modified microchannels. (n=4)

Samples	Flow Condition			Static Condition		
	1×10^8	1×10^6	1×10^4	1×10^8	1×10^6	1×10^4
Average number of adhered cells in unmodified microchannels	16283	6652	547	7078	3021	238
Average number of adhered cells in modified microchannels	818	401	43	595	285	22
Suppression ratio (%)	94.98 ± 0.15	93.97 ± 0.14	92.15 ± 0.51	91.60 ± 0.25	90.56 ± 0.34	90.46 ± 0.65

Appendix B

Water contact angle measurements

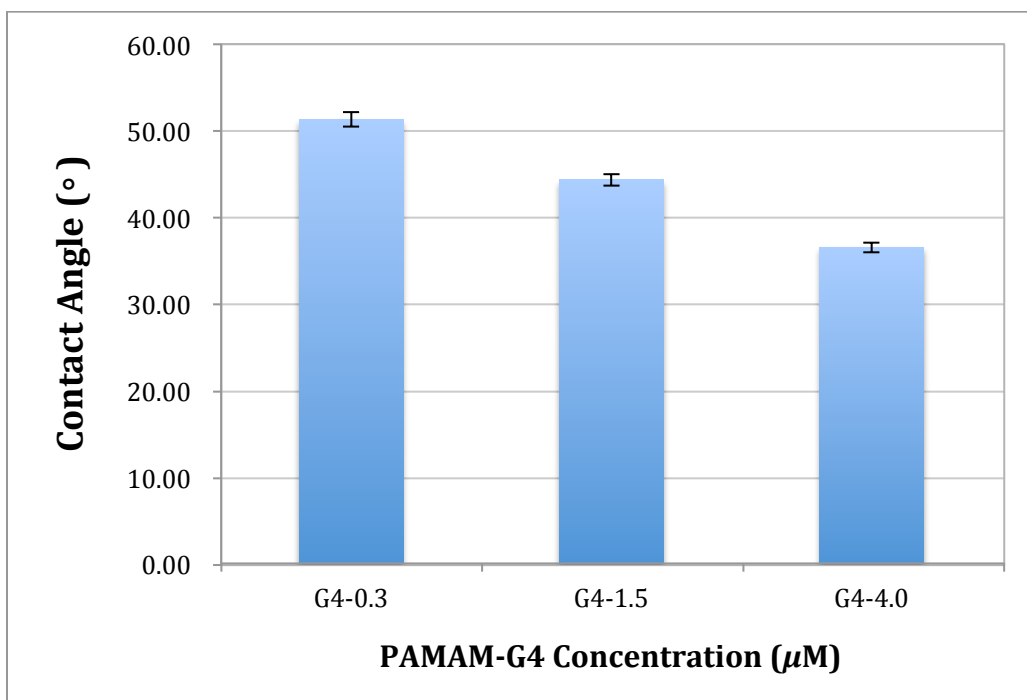


Figure S2. Water contact angles of PAMAM-G4 modified PDMS surfaces using different PAMAM-G4 concentrations. Error bars are standard deviations of the measurements (n=8).

Characterization of surface morphologies

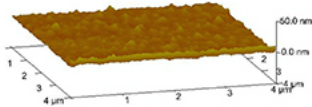
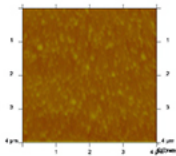
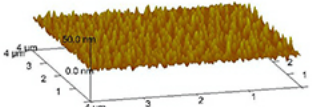
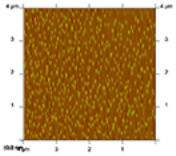
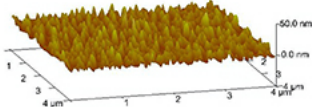
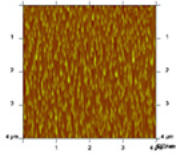
Groups			AFM 3D Image	Vertical View	Roughness
PAMAM Conc. (μM)	G4	0.3			1.38 ± 0.14
	G4	1.5			2.98 ± 0.14
	G4	4.0			3.62 ± 0.23

Figure S3. AFM images and roughness (nm) of PAMAM-G4 modified PDMS surfaces using different concentrations of PAMAM-G4. Error bars are standard deviations of the measurements (n=4).