Development of Membrane in Microfluidic Device for Sorting and Detection for Potential Heterogeneity Investigation

by

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Abstract

Membrane fabrication and integration with microfluidic devices has received increased attention for applications including bio-detection (a device providing analytical information in a selective and quantitative manner using a biological recognition element), membrane-based separation, and biological sample purification. The main challenges associated with these applications have been: 1) meeting sensitivity/selectivity requirements, 2) decreasing costs, 3) maintaining the mechanical stability of the membrane, 4) offering high throughput. Therefore, the main goal of this study was to demonstrate size-based membrane separation and bio-detection using double layer channel developed in our lab and to show how the membrane integrated channel can selectively separate rod shape cell separation with various aspect ratio based on size and enhance bio detection rate with flow. Based on an existing double-channel and cross-flow microfluidics platform, we explored various polymeric materials for fabricating porous membranes to use in pore-size-dependent separation. We induced pores via stop-flow lithography, and investigated membrane properties and limitations for pore-size-dependent separation. We investigated potential applications of poly(ethylene glycol) diacrylate (PEGDA)-based membrane integrated platforms in biological molecule detection based on streptavidin and biotin interaction. We demonstrated that flow and concentrations can enhance target detection in this platform.

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List of Abbreviations

Å Ångström

Biotin-FITC Biotin-4-fluorescein isothiocyanate

Chk1 Checkpoint kinase 1

DNA Deoxyribonucleic acid

FITC fluorescein isothiocyanate

dNTP Deoxyribonucleoside 5'-triphosphates

DSB Double stranded break

DVB Divinylbenzene

G1 phase Gap 1 phase

G2 phase Gap 2 phase

HU Hydroxyurea

K_a Dissociation constant

M phase Mitosis phase

NOA Norland Optical Adhesive

PBS Phosphate buffered saline

PBST Phosphate buffered saline with 5% tween 20

PDMS Polydimethylsiloxane

PEGDA Poly(ethylene glycol) diacrylate

RE Reynolds number

SA-PEG-A Streptavidin-PEG(2000)-acrylate

S phase DNA synthesis phase

ssDNA Single stranded DNA

Strep Streptavidin

St Stokes number

TETA Trimethylolpropane ethoxylate triacrylate

YES Yeast extract with supplements

CHAPTER 1: Introduction

1.1 Overview of Thesis

A membrane is a selective interspatial barrier between two adjacent phases that regulates solute transport between two compartments (Ulbricht, 2006). The integration of membranes in microfluidics has been a topic of growing interest for decades. This is due to versatility of membranes, and its uses in applications such as gas separation (Robeson, 1991, 1999), reverse osmosis (Lonsdale et al., 1965; Meares, 1966), pervaporation (separating mixture of lipid by partial vaporization via membrane) (Paul and Paciotti, 1975), filtration (Baker et al., 1972), cell culture (Dankers et al., 2011; Ni et al., 2011), and bio detection (Gorey et al., 2008). Membranes are manufactured from various naturally-derived and synthetic materials using different fabrication methods (track etching, photopolymerization, etc.) to confer specific properties/functionalities for targeted applications (Heyderman et al., 2003; de Jong et al., 2006; Kelly et al., 2006; Humble et al., 2004). Membrane with pores can be synthesized; however, pore shape and size of membrane is largely restricted by membrane fabrication methods. Several studies reported that the use of membranes in filtration and biosensor applications is limited due to undesired clogging and fouling (unwanted deposition of matter on surface of membrane) (Gorey et al., 2008; Ji et al., 2008; Kuo et al., 2010).

In this study, we explored various materials for membrane fabrication and investigated properties of synthesized membranes with regard to pore shape and size for membrane separation using stop flow lithography. We also investigated potential applications such as cell separation and bio molecule detection of membranes integrated in a pre-developed two-layer microfluidic device based on biotin-streptavidin interaction. In this study, we demonstrated cell sorting using our platform with less the accumulation of un-transported solutes on membranes.

1.2 Microfluidics

Microfluidics is the science of precisely manipulating fluid through miniaturized fluidic operations. The miniaturization of complex processes allows the rapid analysis of targets, reduces sample usage, and lowers cost compared to conventional larger-scale processes. Thus, the microfluidic approach has attracted widespread attention. Microfluidic devices have been widely used as biological tools for the electrophoresis of biological and chemical species (Doyle et al., 2002), polymerase chain reaction (PCR) (Sun et al., 2002), cell culture (Gómez-Sjöberg et al., 2007; Hung et al., 2005; Lecault et al., 2011; Wu et al., 2010), protein separation (Macounová et al., 2001), immunoassays (Hung et al., 2005), and cell sorting (Krüger et al., 2002). Microfluidic diagnostic tools have been developed for sexually-transmitted diseases (Monaghan et al., 2007; Wang and Lee, 2005), malaria (Gascoyne et al., 2004), and HIV (Cheng et al., 2007). These microfluidic devices represent excellent point-of-care diagnostic modules in the absence of larger-scale testing resources or funding (Yager et al., 2006). In the past couple decades, substantial progress has been achieved in microfluidic technologies capable of miniaturizing systems for diverse applications.

Especially, microfluidic approach has been extensively used to develop simple cell sorting platform due to lack of cost-effective method for cell sorting with high selectivity. For example, some study has demonstrated separation of magnetically tagged leukocytes from whole human blood cell in microfluidic channel by creating high magnetic field gradients (Inglis et al., 2004). Other study also has shown shape-based cell separation in microfluidic channel based on the shape dependent lateral inertial focusing equilibrium position (Li et al., 2017). Despite the microfluidic advance in cell sorting, a microfluidic platform to separate cells in a cost-effective and high throughput fashion is still not available.

1.3 Membrane

A membrane is a semi-permeable barrier (de Jong et al., 2006; Mulder, 1996).

Membranes have been used in a wide range of interdisciplinary fields for various applications due to their functional and structural versatility (de Jong et al., 2006; Mulder, 1996). Typically, membranes can be classified into two categories based on their morphology: dense (nonporous) and porous (Figure 1.1) (de Jong et al., 2006).

Transport in dense (nonporous) membranes occurs through diffusion under the driving forces of concentration, pressure, or electrical potential gradient because only single molecules can penetrate dense membranes (Baker, 2004; de Jong et al., 2006). The permeability of component toward membranes is determined by components diffusivity and solubility in the membrane material, indicating that component permeability depends on components interaction with the membrane material (de Jong et al., 2006). Therefore, dense membranes are suitable for applications such as gas separation (permeation) (A. Stern et al., 1974; Gantzel and Merten, 1970), pervaporation (separating mixture of liquid by partial vaporization via membrane) (Liang and Ruckenstein, 1995; Liu and Kentish, 2018), reverse osmosis (Vos et al., 1966).

Unlike dense membranes, transport of solute in porous membranes occurs through empty spaces in the membrane rather than the material itself. Porous membranes can be further classified into three categories depending on pore diameter: microporous structure for nanofiltration (Fritsch et al., 2012; Rios et al., 1996), mesoporous structure for ultrafiltration (Huang et al., 2012; Wu et al., 2012), and macroporous structure for microfiltration (Wang et al., 2012). Porous membranes are suitable for separation applications requiring various pore sizes (Figure 1.1 and 1.2) (de Jong et al., 2006). Thus, with regards to performance in transport of

solute, dense membranes strictly depend on materials, while porous membranes depend on morphologies and materials (de Jong et al., 2006; Mulder, 1996).

Figure 1. 1 Schematics of morphological types of membranes: Non-porous membrane (A) and porous membrane (B). (Adapted from Baker, 2004)

Figure 1. 2 Average pore diameters of membrane required for different filtrations and their range of targets. (Adapted from Baker, 2004)

1.3.1 Various Materials and Membrane Fabrication Methods

Membranes with specific porous morphologies are made from various materials to influence the application, including: (i) inorganic polymer (oxides, ceramics, and metals), (ii) organic polymers, (iii) mixed matrices or composite materials.

Hydrogel is a well-characterized polymeric material used for separation membranes. Hydrogel membranes have been synthesized with immobilized proteins for biological applications including drug delivery, tissue engineering and chemical and biological sensors (Jagur-Grodzinski, 2010; Langer and Tirrell, 2004; Lee et al., 2010). Both synthetic materials (e.g. poly(acrylic acid), polyacrylamide, and poly(ethyleneglycol) (PEG)) and naturally-derived materials (e.g. chitosan, dextran, and alginates) have been used to produce hydrophilic and bio-friendly hydrogels with a three-dimensional mesh structure that specialize in water retention (Lee et al., 2010; Pregibon, 2008). For example, hydrogel PEG material has been successfully used to synthesize membranes with a more hydrophilic surface to prevent biofouling (unwanted deposition of matter on surface of membrane) by minimizing hydrophobic interactions (Bloisi et al., 2007; Gorey et al., 2008; Pregibon, 2008). Also, hydrogels have been used in drug delivery application due to unique water retention properties of hydrogel (Anderson, 1983; Chen et al., 2004; Chu et al., 2004; Hoare and Kohane, 2008; Obaidat and Park, 1997).

Membrane with pores can be synthesized via various fabrication methods such as sol-gel processes and phase separation of polymer (de Jong et al., 2006; Humble et al., 2004; Russo et al., 2004; Ulbricht, 2006). One of conventional method widely used in making membrane with pore is track-etching technique. In this method, chemical bond of thin membrane polymer is broken by perpendicular radiation beam exposure with high energy. Then, the exposed

membrane is etched in chemical bath selectively attacking damaged polymer leading to track etched membrane with pore size ranging from 20 nm to 10 μm . Despite small pore size of track etching membrane, shape of pore and surface porosity are very limited (Wang et al., 2013).

Photo-curable polymeric materials and microfluidic based-photolithography technique called stop-flow lithography (SFL) may also be used to fabricate membranes with specific shapes and porous characteristics (Doyle et al., 2010). SFL is fast, high-throughput, and tunable (Dendukuri et al., 2006, 2007; Helgeson et al., 2011; Pregibon, 2008). Shape and size of membrane is determined by photomask and objective lens and thickness of membrane is determined by height of channel. The porosity of these membranes can be manipulated by photomask feature size for micropores and porogen (pore inducing solvent/inert molecule which contributes to polymer properties) concentration for nanopores, and membranes can be further functionalized for targeted applications (de Jong et al., 2006; Lee et al., 2010).

Fabricated membranes have been combined with microfluidics to investigate new applications. In this approach, membrane-based filtration can be operated in two modes: dead-end mode, or continuous mode (Figure 1.3). In dead-end mode, a stream completely flows through the membrane, leaving the accumulation of unfiltered solutes on the membrane (Figure 1.3). In continuous operations (*e.g.* cross-flow, counter-current flow and co-current flow), the feed main stream (=retentate+ permeate) is divided into two substreams: the substream passing through the membrane (called 'permeate') and the subsequent remaining substream (called 'retentate') (Figure 1.3). In the continuous mode, the desired target products can be attained from either permeate or retentate depending on the application.

Figure 1. 3 Membrane based filtration with two different modes: Dead end mode (A) and Continuous mode (B). Large particles are shown in yellow color and small particles are shown in red color. Black dotted line represents membrane with pores.

1.3.2 Membrane Applications

1.3.2.1 Sorting

Microfluidic-based membrane filtration has been used for the removal of spiked dust and particulate matter aggregates from bacterial spore suspension (Floriano et al., 2005), the removal of solutes such as protein from whole blood (Mohamed et al., 2006), and the separation of cells from whole blood (Li et al., 2016). In this system, solutes smaller than membrane pore size penetrate the membrane, while solutes larger than the membrane pore size are retained (Ulbricht, 2006). Thus, the separation is restricted by the pore size of the membrane (Ulbricht, 2006). For instance, membrane filters with circular pores have been integrated with electrolysis for spiked cancer cell separation in blood and genome analysis with a high-throughput and

almost 90% efficiency based on size difference between circulating tumor cells (CTCs) and blood cells (Zheng et al., 2007).

Yet, size-based membrane separation faces several challenges. Clogging caused by high concentrations of particulate matters leads to local irregular flow (Ji et al., 2008). Deformable rare cells such as circulating tumor cells (CTCs) also can create local perturbations around pores and interrupt size-based membrane separation (Kuo et al., 2010) directly affecting sorting efficiency. Separating these large tumor cells from blood may be challenging because a large volume of whole blood (7.5 mL) is required to obtain valid results for size-based sorting (Cristofanilli et al., 2004).

1.3.2.2 Biosensor

Biosensors have been used for organic and biological molecule detection. Chemo or biosensors consist of a receptor coupled with a transducer and a detector converting chemical signals to physical signals via a receptor binding reaction (Ulbricht, 2006). In detection, the membrane acts as a barrier to protect the receptor from other environmental disturbances for selective access to the receptor. In addition to selectivity, the receptor needs to be immobilized in the membrane matrix (Ulbricht, 2006). Moreover, membranes can be functionalized with immobilized biomolecules for affinity binding (Borcherding et al., 2003), thin-layer molecularly imprinted polymer (MIP) (Kochkodan et al., 2001) or ion-exchange groups (Ulbricht and Yang, 2005) to develop rapid, portable and sensitive biosensors. Although membrane functionalities and morphologies can be diverse depending on the composition (monomer, solvents, etc.), components (base membrane, monomers) and conditions (UV exposure time, etc.), the entire

pore surface of a porous membrane is evenly covered with functionality for precise targeted detection (Ulbricht, 2006).

1.4 Biological Heterogeneity

Heterogeneity is a fundamental building block of a biological system prevailing across all levels of living organisms ranging from bacteria cells to human tissue. Heterogeneity widely refers to variability within any given set of samples and heterogeneity in a cell population can be classified as phenotypic (non-genetic) heterogeneity and genetic heterogeneity in biology. Phenotypic (non-genetic) heterogeneity refers to phenotypic variation of distinct molecular signature in a cell population sharing the same gene indicating that the trait differences are not caused by genetic differences between the cells, whereas genetic heterogeneity refers to genetic variation in cell populations where genomes of individual cells are not completely identical. The heterogeneity of a population reflects cell-to-cell variability in traits measured functionally or morphologically. For example, most tumors contain mixtures of subpopulations and dominant main populations due to genetic heterogeneity within a tumor cell population. The heterogeneous populations interact with each other and affect tumor formation (Lecault et al., 2012). In addition, cancer cell resistance to chemotherapy at a certain time point, but not at another time, has been demonstrated due to switching between distinct phenotypes without genetic change (Hoek and Goding, 2010; Hölzel et al., 2013; Pisco et al., 2013; Quintana et al., 2010; Roesch et al., 2010; Schadt et al., 2009). Understanding heterogeneity within the main population can reveal a complex network of interactions among the subpopulations.

Heterogeneity may also provide vital cues for treatment-induced responses of patients with the same disease such as breast carcinoma (Potts et al., 2012; Wills et al., 2013) . This may help to design personalized therapeutic regimens for diverse conditions because heterogeneous individual cells can reveal different responses in drug resistance and disease progression toward tumor microenvironments (Tirosh et al., 2016). At some level, analysis of heterogeneity has been neglected because conventional methods cannot separate a heterogeneous sub-population from a homogenous main population. Still, it is important to be aware of heterogeneity in samples.

General assumption about cell culture samples is that cells within a cell culture are homogeneous; however, failing to note change in heterogeneity caused by intrinsic noise from variations in DNA (Blainey and Quake, 2013; Eberwine et al., 2013), RNA (Sandberg, 2013), proteins (Spitzer and Nolan, 2016), and metabolites (Zenobi, 2013) may lead to the misinterpretation of measurements (Hosic et al., 2016). Therefore, it is critical to understand functional, compositional, or genetic heterogeneity because even cells with identical functions show different responses under identical environments (Kalisky et al., 2011; Kent et al., 2009; Wang and Bodovitz, 2010; Wu and Singh, 2012).

1.5 Fission yeast, *Schizosaccharomyces pombe*

Schizosaccharomyces pombe, commonly called fission yeast, is a unicellular eukaryote. It has a rod-like shape and is ~7–14 μm in length and ~4 μm wide. *S. pombe* has been used as a model organism to study the eukaryotic cell cycle. The cell cycle is a program of sequential events leading to cell growth and division (Hayles and Nurse, 2018). The cell cycle consists of an initial growth and metabolism phase called growth (also called *gap*) 1 phase (G1), followed

by DNA replication in the synthesis or S phase. A second growth/ *gap2* phase (G2) follows as the cell prepares for chromosome segregation and cell division during mitosis (M phase).

Checkpoints are mechanisms that arrest the cell cycle in response to damage or stress. Generally, checkpoints are found at the major transition points of the cell cycle to prevent progress into the next phase. In the mitotic cell cycle of *S. pombe*, there are four checkpoints. The G1/S transition (called 'Start') in which nutrient is checked controls cell size before committing to a new cycle of DNA synthesis and division. The intra S phase ensures that DNA is replicated stably without any DNA damage. The G2/M transition where DNA is checked for any damage during replication ensures that all the proteins are present for cell division. The metaphase/ anaphase transition in mitosis where sister chromatid segregation (anaphase) is prevented until mitotic spindle is correctly aligned with all the chromosomes (metaphase) (Hayles and Nurse, 2018; Sveiczzer et al., 2004). Cell size of fission yeast is affected by environmental cues such as nutrient conditions and environmental stress. For example, cell division occurs at smaller cell size in poor nutrient condition compared to nutrient-rich media. Under replication stress, Rad3 is activated by molecular sensor, Rad9-Hus1-Rad1 (9-1-1), and activated Rad3 recruits mediator protein Mrc1 and activates Cds1, leading to cell cycle arrest at S-G2 phase until the replication stress is resolved (Figure 1.4) (Lindsay et al., 1998). In the presence of DNA damage, Rad3 is activated by another molecular sensor, Mre11-Rad50-Nbs1 (MRN). Activated Rad3 recruits mediator protein Crb2 and activates Chk1 leading to cell cycle arrest at G2-M phase until DNA damage is repaired (Figure 1.4) (Lindsay et al., 1998). In the presence of the environmental stresses, cell elongation appears in wildtype, *chk1Δ*, *cds1Δ* and *mrc1Δ* strains, whereas cell untimely torn (cut) phenotype with small size appears in *rad3Δ* cells (Tanaka and

Russell, 2001; Zhao and Russell, 2004). Moreover, previous study has demonstrated that polarized growth of fission yeast linked to DNA replication checkpoint (Kume et al., 2011).

S. pombe may be a good model organism to investigate cellular heterogeneity associated with environmental stress induced cell morphologies size-based separation. In particular, *S. pombe* has highly polarized growth patterns with various cell aspect ratios and a defined shape and size which may indicate cell cycle phase (Chang and Peter, 2003; Lew and Reed, 1993). Its similarity to human checkpoint mechanisms and pathways means that this model may be used to investigate different response of heterogeneous individual cells in cancer development and treatment.

Figure 1. 4 Schematic of DNA replication and damage checkpoint pathways in *S. pombe*.

1.6 Rationale and Objectives

Separating heterogeneous cell subpopulation from main cell population based on size and shape has always been challenging due to the lack of simple cost-effective techniques with high selectivity. First objective of this work was to demonstrate size-based separation using the membrane integrated microfluidic platform in order to monitor cell behavior in flow in response to membrane pores corresponding to cell shape and size. Second objective of this work was to apply the membrane integrated microfluidic platform for novel flow dependent bio detection based on biotin-streptavidin interaction. Our membrane integrated platform may provide precise size-based separation and enhanced detection rate since the platform can have flow vertically crossing the installed membrane.

CHAPTER 2: Materials and Methods

2.1 Materials

Poly(ethylene glycol) diacrylate 250 ($M_n = 250$ g/mol, PEG-DA 250, Sigma-Aldrich), poly(ethylene glycol) diacrylate 700 ($M_n = 700$ g/mol, PEG-DA 700, Sigma-Aldrich), Trimethylolpropane ethoxylate triacrylate ($M_n = 428$ g/mol, TETA, Sigma-Aldrich), Norland Optical Adhesive 61 (NOA61, Norland Products), Norland Optical Adhesive 81 (NOA81, Norland Products) and Divinylbenzene (DVB 80%, Sigma-Aldrich) were used for membrane synthesis. 2-hydroxy-2 methylpropiophenon (Darocur 1173, Sigma-Aldrich) was used as a photo initiator for PEGDA and TETA. Phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (Irgacure 819, Sigma-Aldrich) was used as a photo initiator for DVB. Streptavidin (Invitrogen), Succinimidyl carboxy methyl ester (SCM)-PEG (2000)-acrylate (Laysan Bio, Inc.) and 1x phosphate-buffered saline PBS(1x PBS, Gibco™) were used to prepare Streptavidin-PEG(2000)-acrylate (SA-PEG-A) solution for streptavidin conjugated membrane synthesis. Biotin-4-fluorescein isothiocyanate (Biotin-4-FITC, Invitrogen) in deionized (DI) water was used to confirm the streptavidin incorporation into the hydrogel particle networks.

2.2 Microfluidic Device Fabrication

A slit microfluidic channel was fabricated for membrane synthesis. The microfluidic channel was designed with computer-aided design (CAD) software. A micro pattern generator (μ PG 501, Heidelberg Instruments, Heidelberg, Germany) was used to print a patterned photomask onto a glass substrate. A silicon wafer was spin-coated with SU-8 2025 photoresist (Microchem, Newton, MA, USA), and UV light was exposed to the silicon wafer through the

patterned photomask. SU 8 developer solution was used to remove unpolymerized photoresist and develop the positive pattern on the master mold.

To fabricate microfluidic channels, polydimethyl-siloxane elastomer (PDMS, Sylard 184, Dow Corning) was mixed with a curing agent (PDMS, Sylard 184, Dow Corning) at a ratio of 10:1. The mixture was poured onto a SU-8 patterned silicon wafer (SU-8 photoresist, Microchem) and baked in an oven at 65 °C for 1 hour to mold the PDMS mixture with the patterns. As partially cured PDMS coated glass were prepared, this molded patterned PDMS was attached to a partially-cured-PDMS-coated glass, prepared at 65°C for 20 minutes, and baked for another 1 hour to complete the sealing between the molded PDMS and the coated glass. A double layer microfluidic channel was also fabricated by the standard soft lithography method. Design of top and bottom microfluidic channels was drawn with CAD software and printed as patterned photomask on the glass substrate using a micro pattern generator (μ PG 501, Heidelberg Instruments, Heidelberg, Germany). Spin-coated SU-8 2025 photoresist silicon wafer (Microchem, Newton, MA, USA) was prepared and UV light was exposed to the silicon wafer through the patterned photomask. Remaining unpolymerized photoresist was removed from the silicon wafer. The positive pattern master mold was developed using developer solution. The bottom channel master mold was prepared by repeating a SU-8 spin-coat and UV light exposure twice using two different photomasks in mask alignment systems (EVG®620, EVG, Tempe, AZ, USA) to construct a double layer pattern on the silicon wafer. Then, a mixture of polydimethyl-siloxane elastomer (PDMS, Sylard 184, Dow Corning) and a curing agent at a ratio of 10:1 was prepared and poured onto two different SU-8 patterned silicon wafers (SU-8 photoresist, Microchem). The silicon wafers were baked in an oven at 65°C for 30 minutes to mold the PDMS channels. After partial curing, molded top and bottom PDMS channels were peeled off

from the master wafers and aligned using a stereo microscope (Stereo Microscope Z10, Unitron, NY, USA) in multi-layer assembly. The assembled top and bottom channels were then baked at 65 °C for another 2 hours to fully cure the PDMS channels.

2.3 Photopolymerization Setup

The fabrication system for membrane is based on a stop-flow lithography setup. A metal arc lamp (Lumen 200, Prior Scientific, Rockland, MA, USA) was connected to an Axio Observer inverted microscope to provide a UV exposure source controlled by a UV shutter (Lambda SC, Sutter Instruments, Novato, CA, USA). The prepolymer solutions were supplied through a pneumatic tubing system, consisting of a pressure regulator (Type 100LR, ControlAir, Amherst, NH USA) serially connected to a three-way solenoid valve (Model 6014, Burkert, Germany) and a PDMS channel. The UV shutter and the solenoid valve were controlled by a program in Labview (National Instruments, Austin, TX, USA) through a digital controller (NI 9472, National Instruments, Austin, TX, USA) to control the UV exposure and prepolymer flow cycle. A microscope equipped with 10×/0.3 objective (N-Achroplan, Ec plan-Neofluar and korr LD Plan-Neofluar, Carl Zeiss, Jena, Germany) was used as the synthesis platform. The desired UV excitation (300 nm) required by polymerization was attained by filtering the UV light source through a UV filter set (11000v3, Chroma, VT, USA). The UV intensity was 280 mW/cm² after passing the filter set. AUTOCAD 2011 was used to design the transparency photomasks. Photomasks were printed at a resolution of 25,000 dpi (CAD/Art Services, OR, USA).

2.4 Membrane Synthesis

A prepolymer solution consisting of 95% PEGDA 250 and 5% photo initiator (PI, Darocur 1173) was supplied to either slit 250 μm tall microfluidic channel. When the flow was fully stopped, UV exposure at 300 msec time through the designed photomasks and a 10X objective lens were applied to polymerize membrane. The synthesized membrane remains in the prepolymer solution and was collected at the outlet. The membrane was washed with ethanol (95% Denatured Ethyl Alcohol, Fisher Scientific). The above polymerization process was repeated with designed photomasks using other materials with different UV exposure times and channel with different height. To make PEGDA250 membrane with 600 μm thickness, a ~ 605 μm tall channel was used. 95% PEGDA 700 and 5% photo initiator (PI, Darocur 1173) precursor solution was prepared and 300msec UV exposure time and ~ 65 μm tall channel was used for PEGDA 700 membrane synthesis. DVB based Membrane was polymerized from precursor solution that contains 85% DVB (80% purity) and 15% irgacure 819(PI) using 20 sec UV exposure time and ~ 20 μm tall channel. unlike other materials, acetone was used to wash synthesized DVB membrane. For NOA 61 and NOA 81, 100 μm tall channel was used; however, different UV exposure times were used: 800 msec UV exposure time for NOA 61 and 500 msec UV exposure time for NOA 81. TETA-PEGDA Membranes were polymerized from precursor solution consisting of 47.5% TETA + either 47.5% PEGDA250 or PEGDA700 + 5% PI using 125 msec UV exposure time and 65 μm tall channel. To synthesize membranes with defined pores, various photomasks with different pore size were used. Bright-field images of synthesized membranes and channels were captured by a charge coupled device (CCD) camera (Axiocam IC, Zeiss) (Figure 3.1 and Figure 3.2).

For streptavidin conjugated membrane synthesis, prepolymer solution consisting of 60% PEGDA700, 35% PBS (1x, Gibco™) with 0.05% Tween 20 (Polyethylene glycol sorbitan monolaurate, Sigma-Aldrich) (PBST), 5% Darocur 1173 (photoinitiator) was prepared. 10 mg/mL streptavidin (Invitrogen) was mixed in 1× PBS buffer and succinimidyl carboxy methyl ester (SCM)-PEG (2000)-acrylate (Laysan Bio, Inc.) at a mole ratio of 1:1 to prepare Streptavidin-PEG (2000)-acrylate (SA-PEG-A). Then, mixture of SA-PEG-A and the PEG monomer solutions in either 2:8 (v/v) ratio or 1:9 (v/v) ratio was prepared as a final prepolymer solution. 100 msec UV exposure time and 65 µm tall channel were used to synthesize streptavidin conjugated membrane. Bright-field Images were captured by a charge coupled device (CCD) camera (AxioCam IC, Zeiss) and fluorescent images were taken with a Nikon D300s camera (Figure 3.4).

2.5 Membrane Installation (pre-developed)

Each membrane was introduced through the inlet of a pre-developed double-layered microfluidic device. Simple pressure from a syringe was applied to place the membrane into the intended location without chemical or physical bonding.

2.5.1 Sorting Setup

A membrane with 5 x 8 µm pore size was installed in the double layer microfluidic channel. For cell separation, fission yeast cells grown in YES media were manually injected via inlet of the membrane integrated channel. Experimental movies of sorting were recorded using an inverted microscope (Axio Observer.A1, Zeiss, Oberkochen, Germany) with an attached high-speed camera (Vision Research, Wayne, NJ, USA).

2.5.2 Detection Setup

Streptavidin conjugated membrane was installed in the double layer channel and Biotin-4-fluorescein isothiocyanate (Biotin-4-FITC, Invitrogen) at a concentration of 0.001 mg/mL (1.6 μ M) in deionized (DI) water was injected through the inlet of double layer channel with various flow rates. 1.6 μ M of Biotin-4-FITC was further diluted to 1.6×10^{-1} μ M and 1.6×10^{-2} μ M. Then, buffer solution (PBST) was introduced to wash installed membrane. Both Bright-field fluorescent images were taken with a Nikon D300s camera. Image J software was used to calculate mean value of membrane intensity by substituting intensity measured in background (Schneider et al., 2012).

2.6 Yeast Strains and Growth Media

The *S. pombe* strain used in the proposed research is wild type FY436 (*h⁻ ura4-D18 ade6-M216 leu1-32 his7-366*) provided by Susan Forsburg (University of Southern California, Los Angeles, CA, USA). Cells were grown in yeast extract with a supplements (YES) medium at 30°C overnight.

CHAPTER 3: Results

3.1 Determination of Mechanical Properties of the Fabricated Membrane

Membranes were fabricated from various materials using standard stop flow lithography (Figure 3.1). To precisely install the membrane into a double-layered channel, we determined the mechanical properties of a membrane fabricated from various chemical materials such as PEGDA 250, PEGDA 700, DVB, NOA 61, NOA 81, and a mixture of TETA and PEGDA 700. The dimensions of each membrane in the precursor solution after polymerization were measured, and their dimensions in a 5% Tween 20 (Polyethylene glycol sorbitan monolaurate) solution were measured after washing. Then, the swelling ratio of each membrane was calculated by dividing the post-swelling dimension in 5% Tween 20 solution by the pre-swelling dimension in precursor solution to determine the mechanical stability of the membrane.

We confirmed that membranes fabricated from either Adhesive 61, Adhesive 81, or DVB maintain their intended dimensions even in DI water, indicating almost no expansion without any structural deformations (Figure 3.2 d-f). Various swelling ratios of membranes were found in different compositions of either PEGDA 250 or PEGDA 700 in TETA solution (Figure 3.2 g-h), while membranes polymerized from a mixture of 47.5% TERA and 47.5% PEGDA 700 and 5% PI showed a swelling ratio (approx. 0.97) close to 1 with stable mechanical stability (Figure 3.2 h and Table 3.1). Photomasks with various sizes of elongated shapes of pores ranging from $4 \times 10 \mu\text{m}$ to $4 \times 20 \mu\text{m}$ size were designed corresponding to the shape and size of fission yeast and used in membrane fabrication with various materials. However, small feature pore patterns were the most well-defined in membranes fabricated from PEGDA materials (Figure 3.2 a, b and c) compared to other materials despite relatively large swelling ratio indicating that PEGDA

membrane with well-defined pore shape and size is suitable for size-based sorting of rod shape cells with different aspect ratio (Table 3.1).

Figure 3. 1 Schematic of membrane fabrication. PEGDA700 membrane has well-defined 12 x 5 μ m pores shown in an image. The bright-field image was captured by a charge coupled device (CCD) camera (Axiocam IC, Zeiss). Scale bar is 200 μ m.

Figure 3. 2 Membranes fabricated from various materials and their swelling/shrinkage. a) PEGDA250 based Membrane (left) in 95% PEGDA 250 + 5% PI precursor solution. 300 msec UV exposure time and 250 μm tall channel (upper dotted box) were used. Shrinkage of PEDGA250 based membrane in dried state (right) shows edge of the membrane is lifted. b) PEGDA250 based Membrane with 600 μm thickness (upper dotted box) in the precursor solution that contains 95% PEGDA 250 + 5% PI. 300 msec UV exposure time is used. c) PEGDA 700 based membrane polymerized from the precursor solution contains 95% PEGDA 700 + 5% PI. Membrane (left) in the precursor solution and membrane (right) in 5% tween20 surfactant solution. 300 msec UV exposure time and 65 μm tall channel were used. d) DVB based

Membrane polymerized from precursor solution that contains 85% DVB (80% purity) + 15% irgacure 819 (PI)-membrane dimension (left-95.2 μm) in the precursor solution with thickness $\sim 15 \mu\text{m}$ (dotted box) and membrane dimension (right-94.7 μm) in dried condition after acetone washing. (Almost no swelling and no shrinkage in other conditions). 20 sec UV exposure time was used. Photomask with 50 μm pore diameter was used because of long UV exposure time. e) NOA61 based membrane comparison between different conditions (in precursor solution(left) vs. in 5% tween 20 solution (right) showing no swelling. 800 msec UV exposure time and 100 μm tall channel was used. f) NOA81 based membrane comparison between different conditions (in precursor solution(left) vs. in dried condition(right) showing no swelling). 500 msec UV exposure time and 100 μm tall channel were used. g) TETA-PEGDA250 based Membrane polymerized from precursor solution that contains 47.5% TETA + 47.5% PEGDA250 + 5% PI (left- in the precursor solution, right – in 5% tween 20 surfactant solution). 125msec UV exposure time and 65 μm tall channel were used. Thickness of TETA-PEGDA250 membrane in 5% tween 20 surfactant solution is $\sim 50 \mu\text{m}$ thickness (dotted box). h) TETA-PEGDA700 based membrane polymerized from precursor solution that contains 47.5% TETA + 47.5% PEGDA700 + 5% PI (left- in the precursor solution, right – in 5% tween 20 surfactant solution). 125msec UV exposure time and 65 μm tall channel were used. Thickness of TETA-PEGD700 membrane in 5% tween 20 surfactant solution is $\sim 60 \mu\text{m}$ thickness (dotted box). Photomask with 4 x 20 μm pore size was used in a) and b). Photomask with 4 x 10 μm pore size was used in c). Photomask with 50 μm pore diameter was used in d) because of long UV exposure time. Pore size in e) and f) was 5 x 16 μm . Pore size in g) and h) was 4 x 10 μm . Bright-field Images were captured by a charge coupled device (CCD) camera (Axiocam IC, Zeiss). Scale bars are 200 μm .

Table 3. 1 Swelling ratio of membranes fabricated from various materials measured in DI solution with 5% Tween 20.

Chemical materials	Swelling ratio
PEGDA 250	Various swelling ratio depending on composition. e.g.) Swelling ratio of 95% PEGDA250 + 5% Pi: ~0.80
PEGDA 700	Various swelling ratio depending on composition. e.g.) Swelling ratio of 95% PEGDA 700+ 5% PI: ~1.16
TETA	Various swelling ratio depending on composition. e.g.) Swelling ratio of 47.5%TETA+47.5%PEGDA700+5%PI: ~0.97 Swelling ratio of 47.5%TETA+47.5%PEGDA250+5%PI: ~0.74
Adhesive 81, 61	Swelling ratio: ~1.0
DVB	Swelling ratio: ~1.0

3.2 Determination of Sorting

3.2.1 With *S. pombe*

Size-based separation in the membrane-integrated microfluidic channel was determined using cells of various sizes from a culture. The membrane integrated microfluidic platform was prepared by the installation of the membrane into the pre-developed double-layered channel (Figure 3.3 a and b). Then, cell suspension was introduced to the membrane-integrated microfluidic platform. Injected cells were randomly distributed in the flow. I observed motions of the rod-like yeast with various aspect ratio during flow, including tumbling rotational motion. The resulting motions of ellipsoidal cells interrupted membrane-based separation, indicating that separation of the elongated shape of *S. pombe* at a micron size is difficult to achieve with the current channel design (Figure 3.3 and Movie A. 1).

Figure 3. 3 Pre-developed double layer channel a) A schematic of the pre-developed double layer channel which is made by assembly of top and bottom PDMS channels. The bottom channel consists of membrane supporting features and membrane site where membrane is seated. b)

illustration of its membrane installation. Membrane is introduced through inlet and placed to the membrane site) (Adapted from Han and Hwang, 2018).

3.3 Determination of Relative Intensity for Biotin-Streptavidin Detection

3.3.1 Bulk Condition without flow versus In-Channel Condition with flow

Streptavidin conjugated membranes were fabricated using stop flow lithography to investigate biotin-streptavidin interaction-based detection (Figure 3.4). The relative intensities of the streptavidin functionalized membrane toward Biotin-FITC in the PDMS channel with flow and in Biotin solution without flow were measured (Figure 3.5 a-c). The membrane rapidly reaches equilibrium intensity (~37 arbitrary unit (A.U.)) in 7 minutes in the channel condition compared to the membrane in the bulk condition, which displays approximately half of the equilibrium intensity of the membrane in the channel condition (Figure 3.5 c). The relative intensity of membrane in the channel solution or and bulk solution of biotin-FITC is different 1 minute after the introduction of Biotin-FITC. This shows the enhanced sensitivity (~15 A.U.) of membranes installed in the channel condition, which is three times greater than the relative intensity (~5 A.U.) of membranes in the bulk condition (Figure 3.5 c). Moreover, the relative intensities were measured from three different membrane using the membrane integrated platform at fixed flow rate (60 μ l/min) of biotin-FITC solution (Figure 3.6).

Figure 3. 4 Schematic of streptavidin conjugated PEGDA membrane fabrication and interaction between biotin-FITC and streptavidin conjugated membrane. The membrane was submerged in 400 μ l biotin bulk solution at a concentration of 0.001 mg/mL (1.6 μ M) for 120min and washed with PBST. Bright-field Images were captured by a charge coupled device (CCD) camera (Axiocam IC, Zeiss) and fluorescent images were taken with a Nikon D300s camera. Scale bars are 500 μ m.

Figure 3. 5 Membrane based biotin-FITC detection in the PDMS channel with flow vs. in biotin bulk solution without flow. a) schematic of comparison between biotin-FITC detection in channel with flow and biotin-FITC detection in bulk solution without flow. b) In channel

condition, membranes were exposed to biotin-FITC at flow rate: 60 $\mu\text{l}/\text{min}$ (left), whereas membranes were submerged in 20 μl biotin-FITC bulk solution (right). c) Intensity comparison between membrane in channel condition and membrane in bulk solution. Biotin-FITC at a concentration of 0.001 mg/mL (1.6 μM) in DI water was used for both in the channel and in the bulk solution. Intensities were measured after washing with PBST buffer solution at 300 $\mu\text{l}/\text{min}$. Both Bright-field fluorescent images were taken with a Nikon D300s camera. Scale bars are 750 μm .

Figure 3. 6 Membrane based biotin-FITC detection at 60 µl/min flow rate of biotin-FITC solution (1.6 µM). 20% streptavidin conjugated membranes were used. Three membranes (n=3) from different batches were used to measure membrane intensity after washing with PBST buffer solution at 300 µl/min.

3.3.2 With Various Compositions of Streptavidin Conjugated with Membranes

Next, we changed the 2:8 (v/v) ratio of the mixture of the SA-PEG-A solution and PEGDA monomer solution to a 1:9 (v/v) ratio to investigate the effects of the amount of streptavidin conjugated in membranes on the sensitivity of membrane-based biotin-FITC detection with a fixed flow rate (60 μ l/min). Both membranes were polymerized from the 10% streptavidin and 20% streptavidin precursor solution. They reached equilibrium of fluorescent intensity in approximately 7 minutes. However, membranes polymerized from a 10% streptavidin precursor solution exhibited half of the maximum equilibrium intensity of membranes from a 20% streptavidin precursor solution (Figure 3.7). This indicates that the maximum relative intensity of membranes installed in a channel at the equilibrium state is determined by the total amount of streptavidin conjugated in the membrane.

Figure 3. 7 Intensity comparison between 20% streptavidin membrane and 10% strep membrane in channel with fixed flow rate (60 $\mu\text{l}/\text{min}$) of biotin-FITC solution. 20% streptavidin conjugated membrane was polymerized from mixture of SA-PEG-A and the PEG monomer solutions in 2:9 (v/v) ratio. Whereas, 10% streptavidin conjugated membrane was polymerized from mixture of SA-PEG-A and the PEG monomer solutions in 1:9 (v/v) ratio. 100msec UV exposure time and 65 μm tall channel were used to synthesize both streptavidin conjugated membranes. Biotin-FITC at a concentration of 0.001 mg/mL (1.6 μM) in DI waster was used for both 20% streptavidin conjugated membrane and 10% streptavidin conjugated membrane. Intensities were measured after washing with PBST buffer solution at 300 $\mu\text{l}/\text{min}$.

3.3.3 With Various Concentrations of Biotin Injected into the Membrane Integrated platform

To investigate how biotin concentration affects the relative intensity of membrane, biotin solutions were diluted to 0.01 μM and 0.1 μM . Then, the fixed total volume (640 μl) of diluted biotin solutions were introduced to the membrane-integrated platform with fixed-flow rates (160 $\mu\text{l}/\text{min}$) to measure relative intensities. Resulting relative intensities show that a high concentration of biotin with a fixed volume explicitly reveals a higher relative intensity compared to a low concentration of biotin introduced to the platform (Figure 3.8). According to our current result, detection limit of our platform may be 10 nM (Figure 3.8).

Figure 3. 8 Intensity comparison between different concentrations of biotin-FITC. 1.6 μM of Biotin-4-FITC was diluted to $1.6 \times 10^{-1} \mu\text{M}$ (0.1) and $1.6 \times 10^{-2} \mu\text{M}$ (0.01) with DI water. The flow rate of biotin-FITC was fixed at 160 $\mu\text{l}/\text{min}$ and 20% streptavidin conjugated membranes were exposed to biotin-FITC solution for 4min in channel. Intensities of the membrane were measured after washing with PBST buffer solution at 300 $\mu\text{l}/\text{min}$.

Chapter 4: Discussion

4.1. Characterization of Various Membranes

The stop-flow lithography technique uses a photomask which shapes UV light to polymerize the membrane from various photocurable materials. Several photocurable materials which can be polymerized via crosslinking have been suggested (Doyle et al., 2010). Mechanical properties of the crosslinked polymers are determined by crosslink densities (Gent et al., 2001). A fabricated polymer with high crosslink density results in a very rigid structure. Reduced viscosities of polymer are caused by low crosslink densities (Gent et al., 2001). In addition, the practical resolution of polymer fabricated from the stop flow lithography is determined by actual magnification of different objectives and feature sizes printable on the transparency photomask (Dendukuri et al., 2006). The length and straightness of sidewalls of polymer are restricted by the depth of field of objectives (Dendukuri et al., 2006). Also, the height of the PDMS channel and oxygen inhibition layers determine the thickness of fabricated polymer in the lithography (Dendukuri et al., 2006). Thus, membranes with desirable shape and size of pores can be synthesized via SFL.

PEG-based hydrogels have been widely exploited as a crosslinking agent in various applications such as drug delivery systems, tissue engineering (Drury and Mooney, 2003), cell cultures (Tibbitt and Anseth, 2009) and biosensors (Le Goff et al., 2015; Liu et al., 2010; Yan et al., 2009) because of their biocompatible structure, transparent nature and high permeability. In this study, we successfully constructed well-defined pore features on PEGDA membranes via optimizing the membrane polymerization process with a 10x objective lens. The smallest feature width we were able to fabricate without blocking pores was approximately 4 μm . Although it has

been reported that the smallest feature size in PEG-based hydrogel lithography is 3~5 μm (Dendukuri and Doyle, 2009; Dendukuri et al., 2007; Li et al., 2014), the diffusion of free radicals can cause undesirable polymerization and undefined feature formation at this scale (Li et al., 2015). In addition to the actual magnification of 10x objectives used in our system, the practical resolution of feature size on the fabricated membrane is largely limited by the smallest feature size printable on the transparency photomask, 10 μm , due to the nature of the stop-flow lithography technique (Dendukuri et al., 2006).

Synthesized PEGDA hydrogel-based membranes were swollen after the post-washing process, leading to reduction of the pore sizes on the membrane. This water-induced swelling behavior of the hydrogel membrane appears when osmotic forces and ionic repulsion exceed attractive forces in the hydrogel (Das et al., 2006). It has been reported that polymer fabricated from a higher molecular weight of PEG undergoes a great change because of their capacity to absorb more water compared to polymer fabricated from a low molecular weight of PEG (Lee et al., 2008a). However, PEGDA250 based membrane shrinkage was observed in DI water because PEGDA 250 is immiscible in both water and oil. PEGDA 250 consists of 3 PEG repeat units and 2 acrylate groups. The PEGDA250 membrane showed some hydrophobicity compared to hydrophilic PEGDA polymer despite the latter's relatively higher molecular weight (Cruise et al., 1998; Nam et al., 2018). In dried conditions, shrinkage-induced deformation of the PEGDA-based membrane also appeared as a result of a loss of water content (Figure A. 1). This severe deformation can arise from the inhomogeneous(uneven) thickness of synthesized PEGDA membranes caused by deformed channels (Li et al., 2014). Thickness uniformity in the membrane can be achieved using glass supports above the channel within the PDMS to prevent direct effects from deformed channels since thickness of membrane is determined by height of

channel in the SFL (Li et al., 2014). In our study, we demonstrated that the swelling/shrinkage behavior can be controlled by mixing PEGDA with trimethylolpropane ethoxylate triacrylate (TETA). TETA is highly miscible due to the amphiphilic nature of the ethylene oxide repeat unit and high functionality and can minimize swelling in the PEGDA polymer (Herlihy, 2009; Safranski and Gall, 2008).

Norland optical adhesive NOA 61 and NOA 81 are UV-curable materials commonly used as glue for glass surfaces, metals, fiberglass and glass-filled plastics. NOA 61 and NOA 81-based membranes were successfully synthesized using stop-flow lithography. The synthesized NOA-based membranes have shown no swelling behavior due to their great solvent resistance. However, polymerizing NOA 61 and NOA 81 with stop-flow lithography has difficulties in attaining complex features such as pores at the (sub)micron level, although NOA-based polymer with simple and relatively large shapes can be synthesized using stop-flow lithography (Bong et al., 2012). This limitation might be associated with the unintended diffusion of free radicals, leading to undesirable feature polymerization in an undefined and uncontrollable manner (Li et al., 2015). Some studies have determined that the (sub)micron level of feature sizes is achieved in NOA-based polymer using the soft imprinting method (Bartolo et al., 2008).

Another UV curable material we used to synthesize membranes was DVB (Christopher and Anna, 2007; Doyle et al., 2010). Some studies have reported that DVB-based polymer has a non-porous possibly nanopores structure which might be suitable for gas separation or Reverse Osmosis but not for our size based membrane separation (Cheng et al., 1992; Li and Stöver, 1998; Ulbricht, 2006).

4.2. Issues with Sorting

For microfluidic hydrodynamic separation, some basic principles and laws for particle motion inside microfluidic devices need to be taken into account (Wu and Hjort, 2009). Four basic principles of particle motion in laminar continuum fluid can be observed in our platform (Asmolov, 1999; Wu and Hjort, 2009). The first principle of particle motion inside a microfluidic platform is the size-determined distribution, which can be defined by the Reynolds number (Re) of flow and Stokes number (St) of the particle (Shevkoplyas et al., 2005; Wu and Hjort, 2009). In size-determined distribution, the particle behavior in the microfluidic platform can be anticipated because Re defines the relationship between inertial force and viscous force, indicating which force is dominant, and the St of the particle describes how quickly the particle adjusts to changes in the surrounding flow (Wu and Hjort, 2009). For instance, particles with small Re and St will follow the streamline of the flow (Asmolov, 1999; Masaeli et al., 2012).

The second principle is size-determined filtering, which is based on the rigidity of the PDMS wall, the filter membrane, and particles. This principle indicates that rigid particles are unable to pass through rigid gaps smaller than their size (Ji et al., 2008). Although the toughness of hydrogel-based filter membranes varies because of various crosslink densities directly reflecting the degree of swelling depending on different concentrations of polymer and crosslinkers, time-temperature-dependent degradation can negatively affect the mechanical strength of polymer, especially in the PEGDA-based filter membrane (Cha et al., 2014; Khandaker et al., 2016; Suekama, 2014). Hence, well-defined pores, the rigidity of synthesized PEGDA-based membranes and the rigidity of particles all directly restrict the passage of target particles or cells below a critical size.

Some studies have reported that membrane filtration for size-based separation faces several challenges such as clogging, fouling (unwanted deposition of matter on surface of membrane) and the heterogenous size of particles or cells within a population (Gossett et al., 2010). The size-determined displacement principle might be applied to explain particle behavior in our platform because particles might encounter obstacles from clogging and fouling. Subsequently, multiple modes will occur (Carlo et al., 2007; Wu and Hjort, 2009). For example, particles with small momentum (small St) will follow their streamline, whereas particles with large momentum (large St) will be deflected away instead of following their streamline (Carlo et al., 2007). Since our separation approach is based on cross-flow, which is perpendicular to the primary main flow, selected particles or cells of interest continue to be separated, while unfiltered particles or cells continue to stay in the direction of primary flow, leading to less clogging (Gossett et al., 2010).

The size-determined guiding principle might be considered to elucidate particle motion during the transition between the PDMS channel and PEGDA membrane observed in our platform (Ji et al., 2008). The principle indicates that special geometry or shapes can guide the flow. In our platform, small geometry differences between the PDMS channel and PEGDA membrane are likely generated because of the nature of the PDMS channel fabrication technique and stop-flow lithography for membrane polymerization (Han and Hwang, 2018; Wu and Hjort, 2009). For instance, when the height difference between the PDMS surface and fitted membrane is similar to the height of a small particle, the particle will be guided by the height difference (Chen et al., 2008; VanDelinder and Groisman, 2006; Wu and Hjort, 2009). On the other hand, large particles will not be guided by height differences (Chen et al., 2008; VanDelinder and Groisman, 2006; Wu and Hjort, 2009).

Moreover, it is critical to address the shape of target particles to understand particle motion in flow, especially cells or particles with various aspect ratios such as elongated rod-like shapes (Masaeli et al., 2012). Our yeast culture has an ellipsoid shape with various aspect ratios up to approximately 1:5. With various cell aspect ratios determined by their cell cycle state, cell motions observed in our platform can be categorized as in-plane-rotation (rotating around the y-axis), out-of-plane rotation (rotating around the x-axis) and no rotation while cells are moving downstream (Masaeli et al., 2012). In our platform, a mix of the above rotational behaviors in flow appeared because cells are not focused at a low cell Reynolds number. If the flow rate and cell Reynolds number increase, in-plane rotation becomes more dominant than in the other two modes (Masaeli et al., 2012). When the cell Reynolds number of the particle is of order 1 or greater, the following two inertial forces are involved in particle distribution because of the rectangular configuration of microfluidic channels: a shear-gradient lift force and a wall-effect lift force-induced particle migration across the streamline (Bhagat et al., 2009; Carlo, 2009; Carlo et al., 2007; Di Carlo et al., 2008; Masaeli et al., 2012; Segré and Silberberg, 1961). Equilibrium particle positions for ordered distribution, where initially randomly distributed particles are focused, can be measured based on a combination of the two forces (Masaeli et al., 2012). Hence, a more uniform rotational motion and distribution of ellipsoidal particles such as tumbling motion with ordered distribution can be expected in well-focused conditions (Jeffery, 1922; Keller and Skalak, 1982; Masaeli et al., 2012; Pozrikidis, 2005).

In addition to the above, other principles can be applied to further elucidate particle motion in various conditions including various channel geometries and various configurations of outlets and inlets (Wu and Hjort, 2009).

4.3. Detection Sensitivity of the Developed Platform

The above size-based cell sorting approach with a membrane-integrated platform will always have limited applications because of the large variety in the size of the same kinds of cells that exist depending on their environment or their cell cycle state (Wu and Hjort, 2009). In addition, many different types of cells with similar sizes existing in samples make it difficult to separate specific target cells based on their size (Wu and Hjort, 2009). Since our platform allows us to use cross-flow in a controllable and sealed manner, membrane-based filtration and sensing were conducted simultaneously in our platform by functionalizing a semi-permeable hydrogel-based membrane with interactive biomarkers. This may provide a good chance to reach rapid detection for biological or clinical studies due to their medical relevance and relatively simple process.

For detection, we used the developed platform integrated with a streptavidin conjugated membrane and introduced biotin-labeled fluorescein isothiocyanate (FITC) to measure fluorescence signal intensity from biotin-FITC binding to the membrane after the post-rinsing process. The biotin-FITC binding to the membrane was not washed away during the post-rinsing process due to the rapid and strong non-covalent interaction between biotin and streptavidin ($K_d \approx 10^{-15}$ M) (Aslan et al., 2005; Bong et al., 2015; Diamandis and Christopoulos, 1991). In addition, bond formation between biotin and streptavidin is extremely resistant to pH, temperature, organic solvents and other denaturing agents (Diamandis and Christopoulos, 1991). This stable interaction is considered as part of the natural defense mechanism because a streptavidin-biotinylated enzyme complex is involved in CO₂ transfer which inactivates the enzyme, leading to the inhibition of bacterial growth. A completely crosslinked PEG polymer has a pore size of ~ 10 Å and can be manipulated by a PEGDA composition in a precursor

solution. This makes the target penetration limited by the PEG hydrogel-based membrane structure without designed pores. Thus, the porous structure of the PEGDA membrane alone we used is sufficient for penetration of a biotinylated target or streptavidin complex which has a radius on the order of ~5 nm without designed pores (Cruise et al., 1998; Mellott et al., 2001).

The fluorescence intensities we measured from the biotin-streptavidin membrane clearly show that the intensities were dependent on the concentration of streptavidin in the precursor solution and biotin-FITC (Bong et al., 2015; Lee et al., 2008b). Although the maximum fluorescence intensity of the biotin-streptavidin membrane in our study is solely determined by the amount of streptavidin conjugated to the membrane, the amount of biotin can be a determinant for the maximum intensity if the membrane is functionalized with biotin, not with streptavidin (Bong et al., 2015; Lee et al., 2008b; Zhu, 2010). Since our approach relies on cross-flow, measured intensities indicate that the biotin-streptavidin membrane reaches equilibrium rapidly compared to the conventional bulk condition (Bong et al., 2015; Lee et al., 2008b).

Our membrane integrated device has the potential to be used as a versatile platform for rapid fluorescent detection of biotinylated biomolecules (Diamandis and Christopoulos, 1991) such as proteins, carbohydrates and nucleic acid by simply functionalizing membranes in a tunable and controllable manners.

Chapter 5: Conclusion and Future work

This study demonstrates the results of membrane fabrication with regard to pores and membrane usage for separation and detection devices. We integrated membranes into our pre-developed double-layer channel, allowing us to use cross-flow in a vertical manner. In this study, we were able to optimize our process to synthesize a PEGDA-based membrane with well-defined, small pores in a tunable and controllable manner. However, other potential materials can be further explored for porous membrane fabrication that can be installed in our pre-developed double-layer channel. This study is the first of its kind to attempt rod-shaped cell separation based on their size in a membrane-integrated channel running a continuous flow configuration. Although several issues such as tumbling motion and the random distribution of particles are present in our study, these issues can be resolved by increasing the channel length between the inlet to the membrane for more ordered and focused cell distribution and flow rate to achieve uniform tumbling motion of rod-shaped cells allowing us to speculate better sorting can be reflected in future design of platform for better size-based separation. This may allow us to separate cells based on size to further investigate heterogeneous populations of the cells. For detection, we demonstrated that fluorescence intensity measured from biotin-streptavidin conjugated membranes is dependent on the concentration of biotin, composition of streptavidin in the membrane. Moreover, detection rate was increased by flow in the membrane integrated platform without relatively long incubation time (more than 30 minutes). Various flow rates can be investigated to further enhance intensity in a relatively short period of time, leading to a slightly decreased equilibrium time (Sheehan and Whitman, 2005). Although the flow rate dependency may be negligible in some applications, flow rate dependency plays important roles in reaction kinetics, especially in low sample volumes such as protein digestion (Gao et al., 2001;

Sheehan and Whitman, 2005). Detection limit of our platform can be further investigated with more diluted biotin-FITC solution to compete with conventional methods used in biology such as ELISA (limit of detection: ~ 4.4 pg/mL) (Appleyard et al., 2011). Furthermore, samples can be recirculated for enhanced separation or target detection with low concentrations of samples that can be used as a potential point-of-care diagnostic device for low HIV viral load (aM) and low heart attack-related troponin level (fM-nM) in the blood (Chin et al., 2012). Other potential applications of our platform could be simultaneous size-shape-based separation and capture which may enable us to investigate size dependent heterogeneity at single cell level, since double-layer membranes can be fabricated with different pore sizes and functionalities and installed on our platform (Li et al., 2015).

Appendices

Figure A. 1 Deformation of PEGDA 250 membrane. a) membrane in precursor solution b) membrane in dried state after ethanol washing. Precursor solution contains 95% PEGDA 250 and 5% PI. 100 μm tall channel and 300 msec UV exposure time were used. Scale bar is 200 μm .

Movie A. 1 Fission yeast cell sorting in the membrane integrated platform.

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