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Sieve-Through Vertical Flow Platform for Efficient Liquid Exchange in Particle-Based Assays

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KEYWORDS particles, vertical flow, DNA isolation, ELISA, flow through, sieve through

ABSTRACT

Particle-based assays are widely used in many biomedical applications. However, the performance of particle-based systems is often compromised by the carry-over contamination caused by the residual reagents during the liquid-exchange process. We have developed a sieve-through platform that utilizes a porous membrane to sieve out the particles, and an absorbent pad to remove the waste liquid by capillary force. The porous membrane is able to contain the liquid in the reaction chamber, and allows the waste liquid to flow through when it is brought into contact with the absorbent pad. The sieve-through platform is able to effectively remove the waste liquid, thereby achieving a more efficient liquid exchange as compared to the conventional process, and minimizing the carry-over contamination. In this study, we have determined the factors that affect the flow characteristics through the porous membrane on the sieve-through platform. We have shown that the sieve-through platform effectively reduces the carry-over contamination. In addition, we have shown particle-based ELISA on the sieve-through platform for the analysis of proteins and cells. We have further demonstrated the potential of the sieve-through platform for high-throughput analysis by presenting a sieve-array, which allows concurrent analysis of multiple samples in parallel. The sieve-through platform can significantly improve the performance of particle-based systems.

1. Introduction

Particle-based systems have been widely adopted in many preparative bioassays [1-11] and analytical bioassays [12-19]. There are several advantages associated with the particle-based systems. First, particles provide the solid substrate for molecule binding. Target molecules could non-specifically adsorb to the particles, such as DNA adsorption to silica particles. [2, 3] Alternatively, they could specifically bind to the particles *via* the ligand-receptor interactions for complementary hybridization. For examples, antibody-conjugated particles could bind to target molecules in applications such as immunoassays. [20-25] Oligonucleotide-conjugated particles are used in the DNA library preparation for high-throughput sequencing. [26, 27] Secondly, particles with unique properties, such as color, size and charge, are useful tags to label target molecules or cells. For example, fluorescent particles are commonly used as barcodes for multiplexed detection. [14, 15] Thirdly, particles have large surface-to-volume ratio, and therefore a small number of particles would provide sufficient surface area for solid phase reactions. Last, particles are easily dispersed in the liquid, and easily separated from the solution phase by either centrifugal or magnetic force.

A typical particle-based assay requires several liquid exchange steps. After binding to target molecules, particles are first separated from the liquid phase by either centrifugal or magnetic force. [1, 3, 5] Next, a liquid handling device, usually a pipette or a vacuum-loaded aspirator, is used to remove the waste liquid from the particles. After that, a new reagent is added, and the particles are re-dispersed. There are a number of issues associated with this liquid-exchange process that could potentially compromise the performance of particle-based assays. Firstly, it is difficult to completely remove the liquid from the particles. Because of surface tension, a small

amount of liquid would stick to the particle surface. Moreover, the liquid would also get trapped at the interstitial space between particles. The residual liquid would significantly contribute to the contamination even after several rounds of washing. Such contamination is observed in the DNA isolated using silica particles, evidence by its abnormal 260/280 and 260/230 ratios. [28-30] Secondly, while it is relatively easy to add the liquid to particles, retrieving the liquid from particles requires a higher level of hands-on skills. One needs to carefully position the liquid handling device, so that maximal amount of waste liquid can be removed without disturbing the particles. If too much liquid is left in the residue, it would lead to a high level of carry-over contamination. If particles are removed with the waste liquid by accident, it would result in a low yield or a low detection sensitivity.

To address the aforementioned issues, we have developed a capillary-driven vertical flow platform for liquid exchange in particle-based systems. The platform, referred to as “sieve-through”, consists of a reaction unit and an absorbent pad (**Fig. 1**). The key feature of the sieve-through platform is the porous membrane that forms the bottom of the reaction chamber. Despite the fact that it is porous, the membrane is able to contain the liquid in the reaction unit due to surface tension. However, when the membrane is brought into contact with the absorbent pad, the capillary force provided by the absorbent pad would pull the liquid out of the reaction unit through the pores. The pores of the membrane are smaller than the diameter of the particles used in the assay. As a result, particles would be sieved out and retained on the membrane during the removal of the waste liquid through the pores, hence the name “sieve-through”.

The major advantage of the sieve-through platform lies in its ability to effectively remove the waste liquid from particles. The capillary-driven liquid-exchange process by the absorbent pad is able to efficiently wipe off the waste liquid from the particles, thereby reducing the carry-over

contamination. The absorbent pad also provides a passive pumping mechanism for simple fluidic handling. In contrast, the conventional liquid exchange by centrifugal or magnetic force would leave a large amount of liquid on the particle surface as a result of surface tension, leading to a high level of contamination. The sieve-through platform provides a simple way of efficient liquid exchange for particles, especially for non-magnetic particles which are more tedious to handle. Although using the paper for liquid handling, it is significantly different from the paper-based microfluidic platform. Besides passively pumping the liquid via the capillary force, paper microfluidics conducts the analytical assays in the paper matrix.[31-33] In contrast, the sieve-through platform conducts analytical assay using particles as the solid substrate. Furthermore, paper microfluidics is well not suited for preparative assay due to the difficulty in sample elution. Extracted DNA samples are not able to be eluted into the solution. They are collected by cutting the paper and use the paper-bound DNA as the sample input for subsequent analysis.[34, 35]

In this study, we characterized the sieve-through platform and performed both preparative and analytical assays on the platform. We demonstrated particle-based solid-phase DNA extraction and showed reduced contamination of the isolated DNA. We also demonstrated particle-based enzyme-linked immunosorbent assay (ELISA) on the sieve-through platform. The sieve-through platform effectively removed the waste liquid during ELISA, resulting in a low background. In addition, we showed the ability of the sieve-through platform to analyze “natural particles” such as cells using immunoassays. Furthermore, we presented a sieve-array that allowed multiple reactions in parallel, demonstrating great potentials of the sieve-through platform for high-throughput applications.

Figure 1. Sieve-through platform. a) Photograph of the sieve-through prototype. b) The sieve-through reaction unit sealed with aluminum foil. c) Schematic illustration of different components of the sieve-through platform. d) 5- μm particles and e) 10- μm particles on the membrane with 3- μm pores. d) and e) are false-colored scanning electron microscopy (SEM) images.

2. Methods and Materials

Methods and materials are briefly described in this section. Detailed methods are available in the Supporting Information.

The porous membranes were NucleporeTM polycarbonate membrane purchased from Whatman (Cat. No. 111110, 111112, 111113, and 111114). The absorbent pad was cellulose pad purchased from Merck Millipore.

2.1 Device prototype

The sieve-through platform consisted of a reaction unit and an absorbent pad (**Fig. 1**, Supporting Information “sieve through device.sldprt”). The reaction unit was designed using the

SOLIDWORKS (Dassault Systèmes, Villacoublay Cedex, France) and prototyped using the Stratasys 3D printer (Stratasys, Rehovot, Israel) (**Fig. 1a and 1b**). A piece of polycarbonate membrane with micron-sized pores was glued to form the bottom of the reaction chamber using polydimethylsiloxane (PDMS) (**Fig. 1c**). For ELISA on the sieve-through platform, particles with surface-conjugated antibodies were pre-stored on the porous membrane of the reaction chamber and sealed with the aluminum foil (**Fig. 1b**). **Figures 1d and 1e** illustrate polystyrene particles of 5- μm and 10- μm diameters, respectively on the membrane with 3- μm pores.

2.2 Device characterization

We tested the device using the buffers to be used in ELISA and nucleic extraction assays. To measure the flow rate through the membrane, 200 μL of liquid was added to the reaction chamber. The reaction unit was then placed on the absorbent pad. The time taken for all the liquid to flow through the membrane was recorded. The flow rate was calculated and expressed in terms of volumetric flux in $\mu\text{L}/\text{cm}^2\cdot\text{s}$. To measure the hold-up time, 200 μL of liquid was added to the reaction chamber. The reaction unit was placed on a horizontal surface apart from the absorbent pad. The time taken for the liquid to leak out from the reaction chamber through the membrane was recorded. The measurement was stopped if no leakage was observed after 1 h.

2.3 DNA isolation

400 ng of human gDNA (Promega, Wisconsin, USA) in 20 μL water was first mixed with binding buffer and silica magnetic particles (Magnetic Suspension G, Qiagen). After the incubation, the liquid was removed by placing the reaction unit on the absorbent pad. The washing process was done by adding the washing buffer to the reaction chamber, and subsequently removing the waste washing buffer through the membrane using the absorbent pad. In the end, 20

μL of water was added to elute DNA from the particle surface. The eluent was retrieved from the top with a pipette. For comparison, DNA was also isolated in the microcentrifuge tube using the same protocol. To exchange liquid in the microcentrifuge tube, the tube was placed on a magnetic stand (Thermo Fisher Scientific, Massachusetts, USA). To evaluate the purity of isolated DNA, the 260/280 and 260/230 ratios were measured using the Nanodrop ND-1000 UV-Vis spectrometer (Thermo Fisher Scientific, Massachusetts, USA). The concentration of the isolated DNA was measured using the PicoGreen assay (Thermo Fisher Scientific, Massachusetts, USA).

2.4 Particle-based ELISA

A model ELISA assay that detected the alpha-fetoprotein (AFP) was used to demonstrate ELISA on the sieve-through platform. AFP level reflects the liver function. It is considered a tumor marker to help with the diagnosis of liver cancer.[36, 37] The capture antibody (Monoclonal anti-AFP antibody, Arista Biologicals, Pennsylvania, USA) was conjugated to polystyrene particles (Bang's Laboratories, Indiana, USA) via biotin-streptavidin interaction. The detector antibody (Monoclonal anti-AFP antibody, Thermo Fisher Scientific, Massachusetts, USA) was labeled with horseradish peroxidase (HRP). For each reaction, 5 μL of antibody-conjugated polystyrene particles was pre-dried on the membrane. The reaction chamber that contained the pre-dried particles was sealed with aluminum foil (**Fig. 1b**) and stored in low humidity until use.

To measure AFP, the HRP-labeled detector antibody was first mixed with the sample and added to the reaction chamber. After incubation, the waste liquid was removed through the membrane using the absorbent pad. The polystyrene particles were washed twice with the washing buffer. Next, ELISA substrate was added and allowed to develop for 15 min. In the end, sulfuric acid was added to stop the reaction. The AFP concentration was determined by measuring the absorbance

of the developed 3,3',5,5'-tetramethylbenzidine (TMB) using the Nanodrop ND-1000 UV-Vis spectrometer. The value was reported as the 1-mm absorbance at 450 nm. To test the device's ability to analyze whole blood samples, AFP was spiked into whole blood samples reconstituted by mixing serum with packed red blood cells. With membranes of the right pore size, particles would stay on the membrane whereas the red blood cells could squeeze through the pores. The assay procedure is the same as the one with buffer.

For comparison, the particle-based ELISA was also performed in the microcentrifuge tube. All reagents were the same. To exchange buffer, the particles were pelleted by centrifugation at 1200g for 10 min. The supernatant was then removed, and the washing buffer was added.

2.5 CD4⁺ Cell Count

Anti-CD4 antibody (Abcam, Cambridge, United Kingdom) was labeled with HRP using the Lightning-Link® HRP Kit (Innova Biosciences, Babraham, Cambridge, United Kingdom). 40 ng of the labeled antibody was mixed with cells in 100 µL of 1×PBS supplemented with 1% (w/v) BSA, and incubated at room temperature for 10 min. The cells were then added to the reaction chamber. Next, the waste liquid was removed using the absorbent pad, leaving only the cells on the membrane. The pore sizes of the membrane were 3 µm for cells in the buffer, and 5 µm in the case of whole blood samples. Subsequently, the cells on the porous membrane were washed twice with 50 µL of 1×PBS supplemented with 0.05% (v/v) Tween 20. After that, 50 µL of 1-Step™ ultra TMB-ELISA substrate solution was added and developed for 15 min. At the end, the reaction was stopped using 0.16 M sulfuric acid. The cell count was determined by measuring the absorbance of the developed TMB substrate using Nanodrop.

The cell count was also performed with the tagging particles. In such an event, 1 µL of 1% (w/v) anti-CD45 Dynabeads (Thermo Fisher Scientific, Massachusetts, USA) was added to the sample to capture the cells. The rest of the procedures remained the same.

3. Results and Discussion

3.1 Device characterization

The most crucial component of the sieve-through platform is the porous membrane. The membrane must contain the liquid in the reaction chamber for a sufficiently long duration, and it should also allow the liquid to flow through it when it is brought into contact with the absorbent pad.

The pore size has a significant influence on the flow rate. For all three buffers tested, including water, 5% (w/v) BSA in $1\times$ PBS, and 70% (v/v) ethanol, the flow rate increased with increasing pore size (**Fig. 2a**). The membranes with smaller pore size actually had a higher porosity. Based on the estimation using the SEM image, the porosity of 1- μm , 3- μm , 5- μm and 8- μm membranes were 13.9%, 10.6%, 7.6% and 6.9%, and the pore densities were ~ 230000 , 30000, 4000 and 2000 / mm^2 , respectively. The pores could be approximated as circular microfluidic channel of equal length. The flow resistance scales inversely to the 4th order of the pore diameter. For parallel identical channels, the flow resistance only scales inversely to the 1st order of the channel number. When comparing the 1- μm to the 8- μm membranes, the effect of pore size increases the flow resistance by 4096 folds, and the effect of the pore number only decreases the flow resistance by 115 folds. The combined effect caused an increase of ~ 36 fold increases in flow resistance for the 1- μm membrane, despite the fact that it has a higher porosity compared to the 8- μm membrane. The thickness of the membrane might be another factor affecting the flow rate as it determines the length of the micro channel. However, we used the commercially available membranes with fixed specifications, and do not have the capability to fabricate membrane of different thickness to study this effect.

The properties of the liquid would affect the flow rate too. As we increased the concentration of glycerol in the solution, the solution became more and more viscous. As a result, the flow rate decreased with increasing glycerol concentration (**Fig. 2b**). In addition, the polarity of the solvent would also have an effect on the flow rate. Since the membrane was hydrophilic, polar solvent would flow through the membrane more easily. As the ethanol concentration in the water increased, the polarity of the solvent decreased, resulting in decreasing flow rate (**Fig. 2c**). Interestingly, the surface tension of the liquid did not affect the flow rate. No significant change in the flow rate was observed with varying concentration of Tween 20 over 5 orders of magnitude (**Fig. 2d**). However, the surface tension of the liquid greatly changed the hold-up time, defined as the duration that the liquid was contained in the reaction chamber without leaking through the membrane. Going beyond the hold-up time, the liquid would leak through the membrane without the assistance of the absorbent pad. As shown in **Fig. 2e**, as the surface tension of the liquid decreased (*i.e.* increasing Tween 20 concentration), the hold-up time also decreased. For 10% (v/v) Tween 20 on the membrane with 8- μm pores, the liquid would leak through the membrane within ~ 25 sec. For 0.1% (v/v) Tween 20 on the membrane with 3- μm pores, there was no leakage observed within 1 h. Typical concentration of Tween 20 is less than 0.1% in commonly used buffers. Therefore, the membrane was able to hold the buffer in the reaction chamber for a sufficient reaction time.

The particles used were ~ 5 μm in diameter. Membrane pore sizes of 5 μm and 8 μm were too large to retain the particles. Flow rate associated with 1- μm pores was too low. Hence, membrane with 3- μm pores was selected for subsequent experiments. The pores were created by track etching, which sometimes led to overlapping pores. Occasionally, overlapping pores were large enough for the particles to get through, resulting in lower sensitivity. This adverse effect of

overlapping pores was negligible for relatively rigid particles, such as polystyrene particles and magnetic particles, because as long as one dimension of the overlapping pore was smaller than the diameter of the particle, it would capture the particle. However, soft particles, such as cells, could deform and squeeze through the overlapping pores, resulting in sample loss and reduced sensitivity. Therefore, it was helpful to tag cells with rigid particles for analysis on the sieve-through platform.

Figure 2. Flow characteristics of the sieve-through platform. a) The effect of membrane pore size tested with water, 5% BSA in 1×PBS and 70% ethanol (v/v). b) The effect of liquid viscosity tested with membrane of 3-μm pores and glycerol of various concentrations. c) The effect of solvent polarity tested with membrane of 1-μm pores and ethanol of various concentrations. d) The effect of liquid surface tension on the flow rate through the membrane of 1-μm pores tested with water containing various concentrations of Tween 20. e) The effect of liquid surface tension on the hold-up time (in seconds) tested with membranes of 1-μm, 3-μm, 5-μm and 8-μm pores, and water containing various concentrations of Tween 20. The white region indicates no leakage in 3600 sec.

Table 1. The purity and recovery yield of DNA isolated on the sieve-through platform and with the conventional procedure using microcentrifuge tube, n = 3.

Absorbance	260/280	260/230	Yield (%)
Control	1.96±0.05	1.77±0.09	–
Sieve-through	1.80±0.10	1.67±0.07	75.5±8.5
Conventional	2.46±0.23	0.32±0.12	69.0±10.4

3.2 Isolating DNA with high purity on the sieve-through platform

It is well-documented that the DNA isolated using silica particles is heavily contaminated by the chemicals in the binding buffer, evidenced by the poor 260/280 and 260/230 ratios. [28-30] The sieve-through platform was able to obtain high-purity DNA by effectively removing the waste liquid during the binding and washing steps, thereby reducing the carry-over contamination.

Compared to the DNA isolated using the conventional magnetic particle-based procedure in the microcentrifuge tube, the purity of the DNA isolated on the sieve-through platform was significantly higher (**Table 1**). For DNA to be considered pure, the 260/280 ratio should ideally be 1.8, and the 260/230 ratio should ideally be 2.0–2.2. [38] The input DNA, which also served as the control, has a 260/280 ratio of ~1.96, and a 260/230 ratio of ~1.77, which indicated reasonably good quality. The DNA isolated on the sieve-through platform has a 260/280 ratio of ~1.80 and a 260/230 ratio of ~1.67, which were close to those of the control. In comparison, the DNA isolated using the conventional magnetic particle approach in a microcentrifuge tube has a very high 260/280 ratio (~2.46) and a very low 260/230 ratio (~0.32). Both ratios deviated significantly from the optimal range, suggesting poor purity of the isolated DNA. As the contamination would

influence the absorbance at 260 nm and lead to an over-estimation of the DNA concentration, the DNA concentration was best estimated using the PicoGreen assay.[39] The DNA recovery yield on the sieve-through platform was ~75%, and the DNA recovery yield obtained using the conventional approach was ~69%. Both the sieve-through platform and the conventional approach used the same reagents to isolate DNA by particle-based solid-phase extraction. However, we were able to obtain DNA with greater purity with the sieve-through platform. The high purity directly resulted from the ability of the sieve-through platform to effectively remove the waste liquid and achieve efficient liquid exchange during the solid-phase extraction. We believe the sieve-through platform could significantly improve the performance of many existing magnetic particle-based preparative assays.

We performed silica magnetic particle-based DNA isolation from HCT-116 cell line using both the sieve-through platform and the conventional procedure in the microcentrifuge tube. A 10-fold serial dilution of cells ranging from 20 to 20000 cells was used in this study. As in the previous case, the DNA isolated on the sieve-through platform has greater purity compared to the DNA isolated using magnetic particles in the microcentrifuge tube (**Fig. 3a** and Supporting Information **Table S1**). The DNA isolated in the microcentrifuge tube was heavily contaminated and displayed an abnormal absorption spectrum, which resulted in a small 260/230 ratio. The 260/280 ratio was slightly higher due to the RNA content in the cells. The solid phase extraction isolated both DNA and RNA. The contaminants included chaotropic agents and organic solvent, which would inhibit PCR amplification efficiency. The isolated DNA was then analyzed with real-time quantitative PCR. A set of primers and a Taqman probe that targeted the GAPDH gene was used to amplify the DNA (please refer to Supporting Information **Table S2** for primer, probe and synthetic target sequences). Regardless the input cell number, the DNA samples isolated using the sieve-through

platform exhibited lower threshold cycle (Ct) number (**Fig. 3b**). On average, the Ct value for the DNA isolated on the sieve-through platform was ~2 cycles less, indicating higher DNA purity and/or higher DNA yield.

Figure 3. Isolating DNA with high-purity using the sieve-through platform. a) UV absorption spectra of isolated DNA. High absorption at short wavelength was observed in DNA isolated using the conventional procedure in microcentrifuge tube, which resulted in an abnormal 260/230 ratio. b) Real-time PCR analysis of isolated DNA. Smaller Ct value was observed for DNA isolated using the sieve-through platform, indicating higher purity and/or higher yield.

We also performed mRNA isolation on the sieve-through platform using poly(T) conjugated particles. We were able to achieve high mRNA isolation efficiency as suggested by quantitative reverse transcriptase PCR (Supporting Information **Fig. S1a**). The performance of sieve-through platform was only slighter better than the conventional process performed in the microcentrifuge tube (Supporting Information **Fig. S1b**). Since the buffer used for mRNA isolation did not contain high concentrations of chaotropic agents or organic solvent, the carry-over contamination would not inhibit PCR.

3.3 ELISA on the sieve-through platform

The sieve-through platform was able to prevent carryover contamination caused by residual liquid. This would allow a low background to be achieved for ELISA applications.

Compared to the particle-based ELISA performed in the microcentrifuge tube, the background was significantly lower on the sieve-through platform (**Fig. 4a**). Furthermore, the signal from positive samples was lower in the microcentrifuge tube, possibly due to the loss of particles during liquid exchange. We successfully quantified a 2-fold serial dilution of AFP on the sieve-through platform using the particle-based ELISA (**Fig. 4b**). AFP is an important tumor marker commonly used for the diagnostics of liver cancer. [36, 37] The standard curve was fit appropriately with the 4-parameter logistic function with an R-square value of > 0.99 (**Fig. 4c**). The particles used in this experiment have a diameter of 5 μm and the membrane has a pore size of 3 μm .

We examined the effect of particle size on ELISA. Particles of 5 μm and 10 μm in diameter were used to analyze the same serial dilution. Results from both particles agreed reasonably well with each other, suggesting that particle size did not have a strong influence on the ELISA (**Fig. 4b**). However, the amount of capture antibodies on the particles would affect the outcome of the ELISA. With 2 μg of capture antibody per mg of particles, the dynamic range of the assay would cover the entire serial dilution. If the loading of capture antibody was reduced to 0.8 μg per mg of particles, the signal decreased significantly for samples containing high concentration of AFP (**Fig. 4d**). In fact, the signal would decrease with increasing AFP concentration due to “Hook’s effect”. [40]

The sieve-through platform was able to perform ELISA directly with whole blood samples. We spiked AFP into whole blood, which was reconstituted by mixing packed red blood cells and

cultured white blood cell line (Jurkat cells) with serum. The reconstituted blood contained 800 white blood cells per 1 μ L of blood with a hematocrit of 45%. 10 μ m-sized particles and membrane with 5- μ m pores were used to allow red blood cells to flow through this membrane. Although white blood cells might remain on the membrane, they are colorless and did not interfere with the signals. The results obtained from whole blood samples matched well with the results from samples in the buffer, suggesting that the sieve-through platform was capable of detecting targets directly from the whole blood (**Fig. 4e**).

Figure 4. Particle-based ELISA on the sieve-through platform. a) Comparison between particle-based ELISA performed on the sieve-through platform and with the conventional procedure in the microcentrifuge tube. The sieve-through platform gives a lower background. b) ELISA standard curve for AFP measured with 5- μ m and 10- μ m particles on the membrane with 3- μ m pores. c) The ELISA standard curve is fitted with a 4-parameter logistic function. d) The effect of particle antibody loading on the ELISA outcome. e) The detection of AFP from whole blood.

3.4 Analysis of "natural particles" on the sieve-through platform

The sieve-through platform operates by separating particles from the liquid based on their size using the porous membrane as the sieve. The same strategy is applicable to naturally existing particles such as cells.

As a proof of concept, we attempted CD4⁺ cell count on the sieve-through platform. CD4⁺ T-cell count was an important marker of the immune system, and was often used for the prognosis of individuals diagnosed with AIDS. [41] To perform CD4⁺ cell count on the sieve-through platform, cells were first tagged with HRP-labeled anti-CD4 antibodies. The cells were then separated and washed on the sieve-through platform. Next, the cell quantity was determined by measuring the absorbance of developed TMB. With increasing amount of input CD4⁺ cells, we observed a corresponding increase in the absorbance (**Fig. 5a**). The signal plateaued for samples with a high cell count.

We also tested the effect of membrane pore size. Membranes with different pore size (1 μm , 3 μm , 5 μm and 8 μm) were used to quantify the CD4⁺ cells. For all four types of membranes, the signal decreased with decreasing cell input (**Fig. 5b**). The signals from membranes with 3- μm and 5- μm pores were at about the same level. The signals from membrane with 8- μm pores were consistently the lowest because the pore size was too large to retain the cells in the reaction chamber. The signals from the 1- μm membrane were also lower than those from the 3- μm and 5- μm membrane. This result was a bit counter-intuitive. One would expect the membrane with smaller pore size to capture more cells, hence resulting in higher signals. We speculated that the small pore size might have induced higher shear stress that mechanically lysed the cells, leading to the low cell count.

By introducing tagging particles to the cells, we could significantly improve the cell count on the sieve-through platform. As a result, we were able to quantify the cells with a wider dynamic range and a better linearity (**Fig. 5c**). In both cases, the negative controls remained clean. The tagging particles recognized CD45 on the cell surface. They did not interfere with the target CD4 antigen. Although they were larger than the pores, cells were highly deformable and might squeeze through the pores. The tagging particles, on the other hand, were rigid and unable to squeeze through the pores. As a result, the binding of the tagging particles to the cells would make it more difficult for the cells to squeeze through the membrane (**Fig. 5d**). We note also that the pores on the membrane sometimes overlapped to form a large pore (**Fig. 5e**), and cells could get through those pores more easily. The tagging particles increased the size of the cells and prevented them from getting through those overlapping pores. Furthermore, the tagging particles captured the lysed cell membranes. These lysed cell membranes retained on the sieve-through platform would generate signal.

We applied the tagging particles to measure CD4⁺ cells in whole blood on the sieve-through platform. The red blood cells would flow through the membrane after the washing. However, the whole blood still strongly interfered with the cell count. Although a positive correlation between the TMB absorbance and the cell count was observed, the signal was weaker than that for the CD4⁺ cells in 1×PBSB buffer (**Fig. 5f**). We speculated that the presence of a large amount of red blood cells would block the pores and induce a higher shear stress, lysing more CD4⁺ cells. In addition, the presence of red blood cells might interfere with the binding of antibodies to CD4⁺ cells.

Figure 5. CD4⁺ cell count on the sieve-through platform. a) Direct quantification of CD4⁺ cells on the sieve-through platform. b) The effect of the pore size on the cell count on the sieve-through platform. c) The effect of tagging particles on the cell count on the sieve-through platform. d) False-colored SEM image of the CD4⁺ cells with tagging particles on the porous membrane. e) SEM image showing overlapping pores. f) CD4⁺ cell count from whole blood vs. 1×PBSB (1×PBS supplemented with 1% (w/v) BSA).

3.5 Sieve-array: potential high throughput applications

The sieve-through platform can be easily scaled up without adding complexity to the fluidic handling mechanism. As shown in **Fig. 6a**, sieve-through reaction units were aligned in an array that allowed us to perform multiple reactions in parallel. The liquid-exchange process on the sieve-array took place at the same time using a large piece of absorbent pad that covered all the reaction units. It was more convenient to handle an array than individual reaction units when analyzing a large number of samples. Furthermore, the sieve-array also reduced the time required to perform the liquid-exchange through parallelization.

As shown in **Fig. 6b**, a serial dilution of AFP was measured on the sieve-array concurrently. The TMB signal decreased with decreasing AFP concentration. Although we only demonstrated a 3×4 array, sieve-arrays of higher density would be plausible by reducing the size of the reaction units and the spacing in between. We believed the sieve-through platform has great potential for high-throughput applications. Our future development plan includes integrating the sieve-through device with sensing modality and automated liquid handling methods for sample-to-answer analysis.

Figure 6. Sieve-array. a) Photograph of the sieve-array prototype. b) Concurrent analysis of multiple samples on the sieve-array for potential high-throughput applications.

4. Conclusions

In conclusion, we have developed a novel sieve-through vertical flow platform that significantly improved the performance of particle-based systems for both preparative and analytical bioassays. The platform utilized a porous membrane to contain the liquid in the reaction chamber. The membrane enabled the liquid to flow through once it came in contact with the absorbent pad. The combined use of the porous membrane and the absorbent pad allowed for effective removal of the waste liquid by capillary force, thereby minimizing the carryover contamination caused by the residual liquid. As a result, we were able to isolate DNA with high purity on the sieve-through platform using particle-based solid-phase extraction. We have also performed particle-based ELISA on the sieve-through platform to analyze proteins and cells with reduced background and greater signal-to-background ratio. In addition, we have demonstrated the high-throughput

potential of the sieve-through platform with a 3×4 sieve-array that allowed for parallel processing of multiple samples.

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Author Contributions

Y.Z and J.Y.Ying conceived the idea and oversaw the project. Y.Z., D.L and A.F. performed the experiment and analyzed the data. All authors wrote the manuscript.

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Notes

Supporting Information available online.

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