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Serial Shear Selection Platform to Enrich Memory B Cells

By

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THESIS

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of the

UNIVERSITY OF CALIFORNIA, DAVIS

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2026

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ABSTRACT

Memory B cells are antigen-specific lymphocytes generated in response to infection (e.g., viral) that provide long-lasting protection against reinfection. Despite lower avidity compared to the total memory B cell population, they can respond to variants of the same virus and, through cross-reactivity, may also contribute to the neutralization of novel viruses. However, current methods for assessing adaptive immune responses have limited ability to determine whether an individual possesses protective immunity against viral infection and frequently fail to detect these low-avidity populations. To address this limitation, we developed an avidity-based serial fluid shear selection strategy. In this method, a series of microfluidic channels are arranged sequentially with increasing widths, generating progressively decreasing shear stresses along the fluidic path. This serpentine design enables the separation of cells based on binding avidity, capturing highly shear-resistant (high-avidity) cells in early regions and progressively isolating less shear-resistant (low-avidity) cells in downstream regions under controlled shear conditions. The device was functionalized with biotinylated hemagglutinin (Biotin HA) for antigen specific interactions against hybridomas specific to influenza or B cells from mice infected with influenza. Our results show that cells with the highest binding avidity exhibited highest capture efficiency at higher shear conditions while cells with lower binding avidity were primarily captured in channels with lower shear stresses. Nonspecific cells maintained a low capture efficiency. Finally, our limited studies in mice infected with the H1N1 flu virus suggest that as many as 29% of B cells in the spleen 45 days post exposure demonstrate specificity to hemagglutinin. These results demonstrate that our platform provides a promising approach for capturing the full spectrum of B cell avidities, thereby enabling a more comprehensive assessment of functional immune responses to evolving viral infections.

INTRODUCTION

The adaptive immune system serves as a highly specialized defense mechanism against foreign particles, including viruses¹. The adaptive immune system generates antigen-specific responses and stored genetic material from the antigen for further protection from future infections. Adaptive immune cells consists primarily of T cells and B cells. B cells recognize antigens through their B-cell receptors (BCR) that are expressed on the surface². B cells originate and mature in the bone marrow³. When activated, B cells can either mature into plasma cells or memory B cells^{1,4}.

Plasma cells produce circulating antibodies that offer immediate protection, and can neutralize viruses directly⁵. Memory B cells do not secret antibodies but persist long-term and can rapidly reactive upon reinfection^{5,6}. Antibodies may decline over time, but memory B cells can remain active for years. Upon re-exposure, they rapidly proliferate and generate new plasma cells and antibodies.⁷ As viruses mutate, older antibodies may no longer bind to the associated antigen as strongly. However, due to the cross-reactivity nature of memory B cells, weaker binding may occur with newer variants of the virus and thus continue to provide immunity^{7,8}.

Viruses consistently mutate, thus altering antibody recognition⁹. Antibodies recognize the specific antigens on the virus, generally proteins^{10,11}. For influenza, this can include the surface protein hemagglutinin (HA). However, as a virus mutates, the epitopes change, thus impacting an antibody's ability to bind to the associated antigen. Circulating antibodies represent past immune responses and may not provide protection against new variants^{7,8}. However, memory B cells are diverse and can generate a functional immune response to new virus variants^{12,13}.

Traditionally, it is assumed that high-avidity B cells are better than low avidity cells to initiate a functional immune response¹⁴⁻¹⁶. High-avidity cells contain highly optimized antibodies that can bind strongly to the previous antigen^{1,17}. While this feature leads to more effective immune responses, high-avidity memory B cells do not necessarily provide broader immunity when viral mutations emerge^{7,8}. Low-avidity memory B cells bind weakly to the previous antigen, but may uniquely be able to recognize new variants and thus contribute to broader immunity^{12,13}. Although viruses constantly mutate, a conserved region of the virous is maintained^{9,10}. The cross-reactive nature of low-avidity memory B cells allows for binding and detection of the mutated antigen^{7,8,13}. For this reason, low-avidity cells may still offer protection despite their weak binding strength to the previous antigen^{7,8,12}.

Current techniques are limited in their ability to accurately detect signs of immunity present. Most methods measure antibodies as a metric in determining immunity as they are easily quantifiable and are present in the peripheral circulation^{18,19}. Immunological assays, such as ELISA, measure the number of antigen-specific antibodies present, but do not measure memory B cell diversity¹⁸. Unfortunately, antibodies provide short-term protection, as antibody levels decline over time²⁰. Memory B cells, however, provide long-lasting protection and can evolve continuously. For this reason, focusing primary on antibody detection rather than on memory B cell diversity can lead to an incomplete characterization of our immune system's ability to respond to a new virus. Other methods, such as flow cytometry, can detect the presence of antigen-specific B cells but rely on equilibrium binding and are thus biased towards detecting high avidity clones²¹.

We have developed a microfluidic device, consisting of a gradient of fluid shear stresses, that has the potential to separate and isolate a mixed B cell population based on binding avidities. The device is functionalized and coated with biotinylated hemagglutinin (Biotin HA), a glycoprotein expressed on the surface of the antigen associated with influenza. Cells are perfused into the device and experience a range of controlled forces (shear stresses) at the HA-coated surface. We hypothesize that cells expressing higher avidity BCRs will preferentially adhere to the channels with the highest shear stress compared to cells with lower avidity BCRs. If the cells encounter different shear forces in series, the device could provide a means to separate or enrich the population of B cells based on BCR avidity.

MATERIALS AND METHODS

3.1. Mice

This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) at UC Davis. C57BL/6 mice were infected intranasally with influenza A/PR8. At designated time points post-infection, spleens were harvested, and single-cell suspensions were prepared. B cells were enriched by CD19-positive selection.

3.2. Hybridomas

Four hybridoma cell lines (H36, H35, H143) were derived and utilized to assess the binding avidity of antigen-specific B cells to the associated influenza hemagglutinin. DS.1 does not express B-cell receptors specific to hemagglutinin and will serve as a negative control. Hybridoma cell lines were placed in cell culture with media consisting of 500 mL RPMI 1640, 10% heat inactivated fetal bovine serum (HI-FBS), and 1% penicillin-streptomycin (Pen-Strep) (Gibco™ 15140122).

3.3. Serpentine Device Design

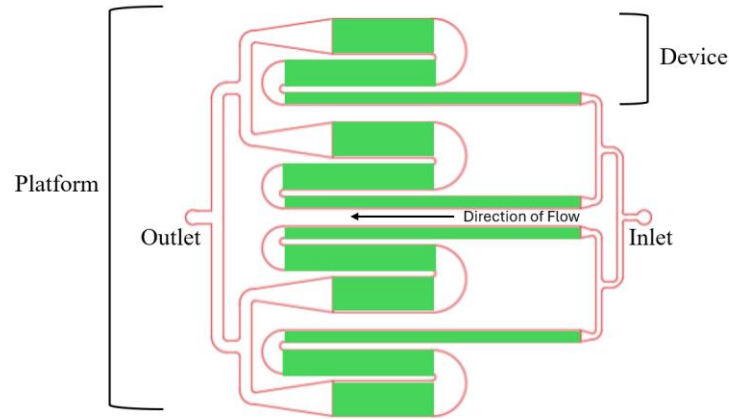


Figure 1.: Serpentine device design. Cells that are perfused flow from the inlet to the outlet through channels of ascending widths (800, 1600, and 2400 μm) and descending shear stresses (0.09, 0.06, and 0.03 dyn/cm^2). The sections of the device noted in the green are sections used for analysis. However, the cells adhered in the manifold of the inlet sections and the curves prior to entering each channel were included in the cell count for analysis.

The serpentine device mold was 3D printed by the UC Davis Tech Foundry Lab. The device consists of a single channel of increasing width and decreasing length the create three different ranges of shear force (**Fig. 1**). The same surface area, volume, and residence time remain constant with each shear force region. The width of the channels are 800, 1600, and 2400 μm . The shear stresses exhibited by this device are 0.03, 0.06, and 0.09 dynes/cm^2 respectively when the inlet flow is 80 $\mu\text{L}/\text{hr}$. These shears were established as previous findings demonstrated significant isolation of high-avidity cells (H36) when perfused with other hybridoma cell lines at a shear stress of 0.06 dyn/cm^2 ²². Four replicates of the device (channels) were connected to form

a platform, through the manifolds in the inlet and outlet section of the platform to create four replicates and thus higher throughput of cells during perfusion while maintaining equal distribution of cells through each device.

3.4. Device Functionalization

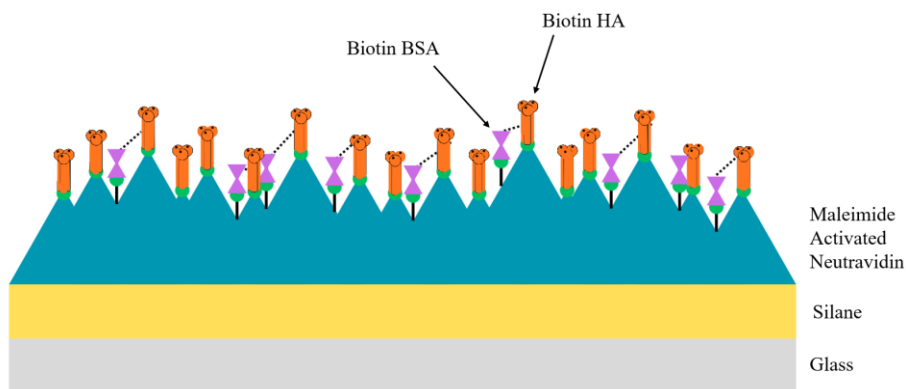


Figure 2.: Schematic of the coating protocol for device functionalization. Protocol involves coating the device with silane, maleimide activated neutravidin, and a Biotin HA-Biotin BSA combination. 13 ug/mL Biotin HA was spiked with 100 ug/mL Biotin BSA so Biotin BSA can attach to exposed hydrophobic regions from Neutravidin.

The device was prepared using polydimethylsiloxane (PDMS; Dow Corning, Midland, MI). Once the PDMS was polymerized, it was peeled off and plasma bonded onto a glass slide (Thermo Fisher Scientific). On day one, plasma treatment was used on the device to oxidize the PDMS surface, thus creating silanol ($-\text{SiOH}$) groups²³. Silane covalently binds to the silanol group to form siloxane bonds, and the device is placed in a desiccator for one hour²⁴. Maleimide activated neutravidin is added in place of a separate GMBS-Neutravidin solution to reduce nonspecific binding by minimizing hydrophobic reactions while preserving its crosslinking nature to the specified antigen²⁵. The platform is left to incubate at room temperature. On day

two, 13 ug/mL of biotinylated hemagglutinin (Biotin HA) is spiked with 100 ug/mL biotinylated bovine serum albumin (Biotin BSA) and introduced to the platform. Biotin BSA was introduced as a blocking reagent to further minimize nonspecific binding by binding to remaining exposed hydrophobic regions from Neutravidin²⁶ (**Fig. 2**). On day three, casein is added as the final blocking reagent to block smaller exposed regions, and device is incubated at room temperature for one hour²⁷.

3.5. B Cell Culture and Staining

Using the Invitrogen Countess 3 Automated Cell Counter, hybridomas cell lines were extracted from the main cell culture to obtain an initial count of 3 million cells. Hybridoma and mouse cells were stained with either CellTrackers CMFDA (green), Deep Red, CMMTR (orange), or BMGC (violet) and left to incubate for thirty minutes. Cells were washed twice, and a second and final count was performed to allocate a finalized desired concentration.

3.6. Cell Perfusion

A 5 ml syringe was filled with 10% BSA and was attached to a flexible plastic tubing using a 23G needle. The 200 ul pipette tip was removed from the outlet and the end of the tubing was placed to attach the syringe to the device. The syringe was placed in a Harvard Apparatus syringe pump. The setting was placed on withdraw only. Initial washing occurred for 20 minutes at a flow rate of 200 ul/hr with 10% BSA to cover remaining exposed regions from the device coating. 10% BSA is removed from the pipette in the inlet and replaced with 200 ul of 1 million cells/mL mixed cell population. Perfusion occurred for 1 hour as a flow rate of 80 ul/hr to

maintain the intended shear stresses of 0.03, 0.06, and 0.09 dynes/cm². The cells are removed from the pipette in the inlet and replaced with 200 uL of 10% BSA for a washing time of 2 hours at a flow rate of 80 ul/hr. Images are acquired using FV1200 Fluoview confocal laser scanning microscope (Olympus).

3.7. Analysis of Device Coating and Cell Binding in Device

Analysis of cell count on the device was conducted using ImageJ. A freehand line was drawn through the center of each channel on a single device. The plot profile plugin was utilized to measure the intensity of FITC Biotin that was present throughout the device after each hour.

Analysis of cell count on the devices were also performed using ImageJ. After brightness, contrast, and threshold were optimized for the images, a particle count was conducted. To prevent inaccurate cell count, manual selection of sections of the device was given to achieve a cell count for each channel, curvature, and inlet sections of the device.

The number of cells that adhered to each channel is useful in determining the capture efficiency and fold enrichment of the cells in the channel. The capture efficiency is the number of specific cells that adhered to each channel normalized by the total number of specific cells perfused. The capture efficiency is calculated separately for each cell type. The fold enrichment is the fraction of specific cells that are captured normalized by the fraction of specific cells that were perfused at the inlet.

RESULTS

4.1. FITC Biotin exhibited unformed coating and intensity when spiked with 100 ug/mL

Biotin BSA

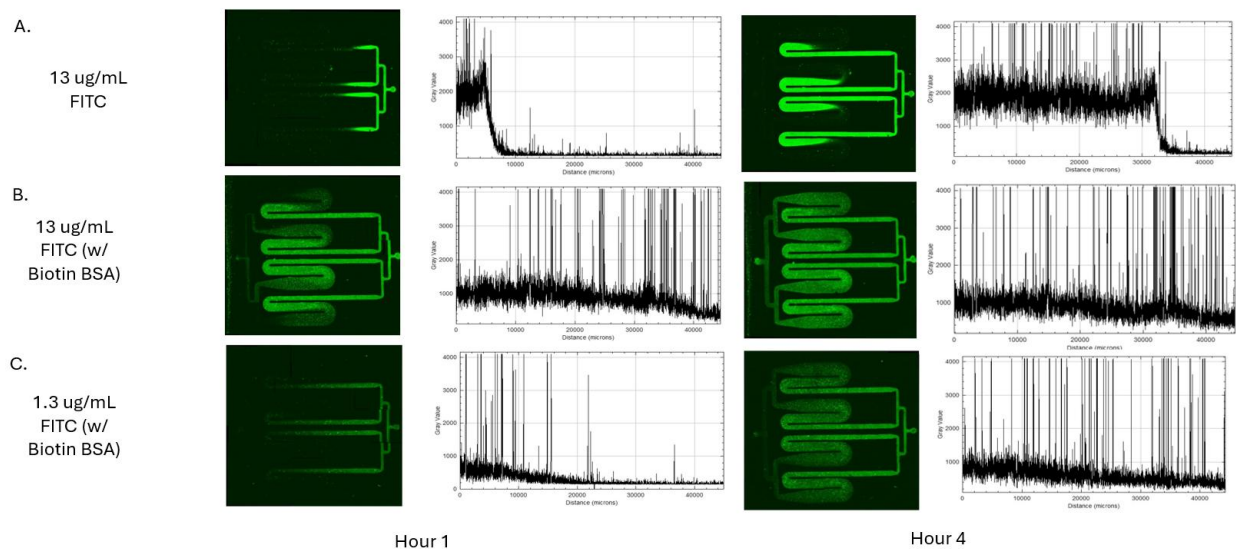


Figure 3.: Serpentine device was coated under three conditions to demonstrate uniform distribution. The devices were coated with 13 ug/mL of FITC Biotin (a), 13 ug/mL FITC Biotin with Biotin BSA (b), and 1.3 ug/mL of FITC Biotin with Biotin BSA (c). A profile plot was generated after each hour in each condition using ImageJ to identify intensity. Images were generated after each hour. An intensity profile was created to quantify the intensity (a.u.) fluorescence present within a single device. Both the images and intensity profiles create a comprehensive assessment on uniform coating made with comparisons of intensities at hour 1 and hour 4.

The device was introduced to hydroxyl ions and coated with 200 uL of 3.65% silane solution and placed in the desiccator for one hour before adding 250 uL of 1000 ug/uL maleimide activated neutravidin. Recirculation occurred twice to ensure even coating throughout the device and the device is placed overnight in the 4C fridge overnight. On day two, the devices were tested for uniform coating using four conditions. In the four conditions, 250 uL of FITC was added to the device and incubated at room temperature for 1 hour. The FITC was then aspirated and replaced with PBS to wash any remaining residuals for 20 minutes. Images were captured

using the confocal microscope. The experiment was repeated on the same device three more times. An intensity profile was created to quantify the intensity (a.u.) fluorescence present within a single device.

In the first condition, one device was coated with 13 ug/mL of FITC biotin. At hour one, little fluorescent is detected on the first channel, with FITC Biotin saturated near the inlet and an intensity of approximately 2000 a.u. This intensity is maintained throughout the device in the four hours this experiment was conducted. At the end of hour 4, intensity remained at approximately 2000, with no fluorescent detected on the third channel (**Fig. 3A**).

In the second condition, another device was coated simultaneously with 13 ug/mL FITC biotin and 87 ug/mL Biotin BSA. At hour one, FITC Biotin is detected through the first and second channel with an intensity of approximately 1000 a.u. At hour two, FITC Biotin is slightly detected in the third channel, but similar intensity is maintained at approximately 1000 a.u. throughout the three channels in hours three and four. This profile plot demonstrates uniformed coating of FITC Biotin throughout the device. Biotin BSA binds to the exposed hydrophobic regions placed by maleimide activated neutravidin, thus allowing for FITC Biotin to bind to maleimide (**Fig. 3B**).

In the third condition, a third device was coated simultaneously with 1.3 ug/mL FITC and 87 ug/mL Biotin BSA. The intensity of FITC Biotin remained below 1000 a.u. in the four-hour duration of the experiment. FITC Biotin was detected in only channel 1 after the first hour, and was slightly detected in channels 2 and 3 after hour channel. The profile plot is consistent with decreasing intensity in each channel, demonstrating uneven coating throughout the device.

Based on the results of the three conditions, we concluded that Biotin BSA at a concentration of at least 87 $\mu\text{g/mL}$ was necessary for the coating process to ensure uniform coating throughout the device (**Fig. 3C**).

4.2. Capture Efficiency and Fold Enrichment of 7.5% specific cells, 92.5% non specific cells

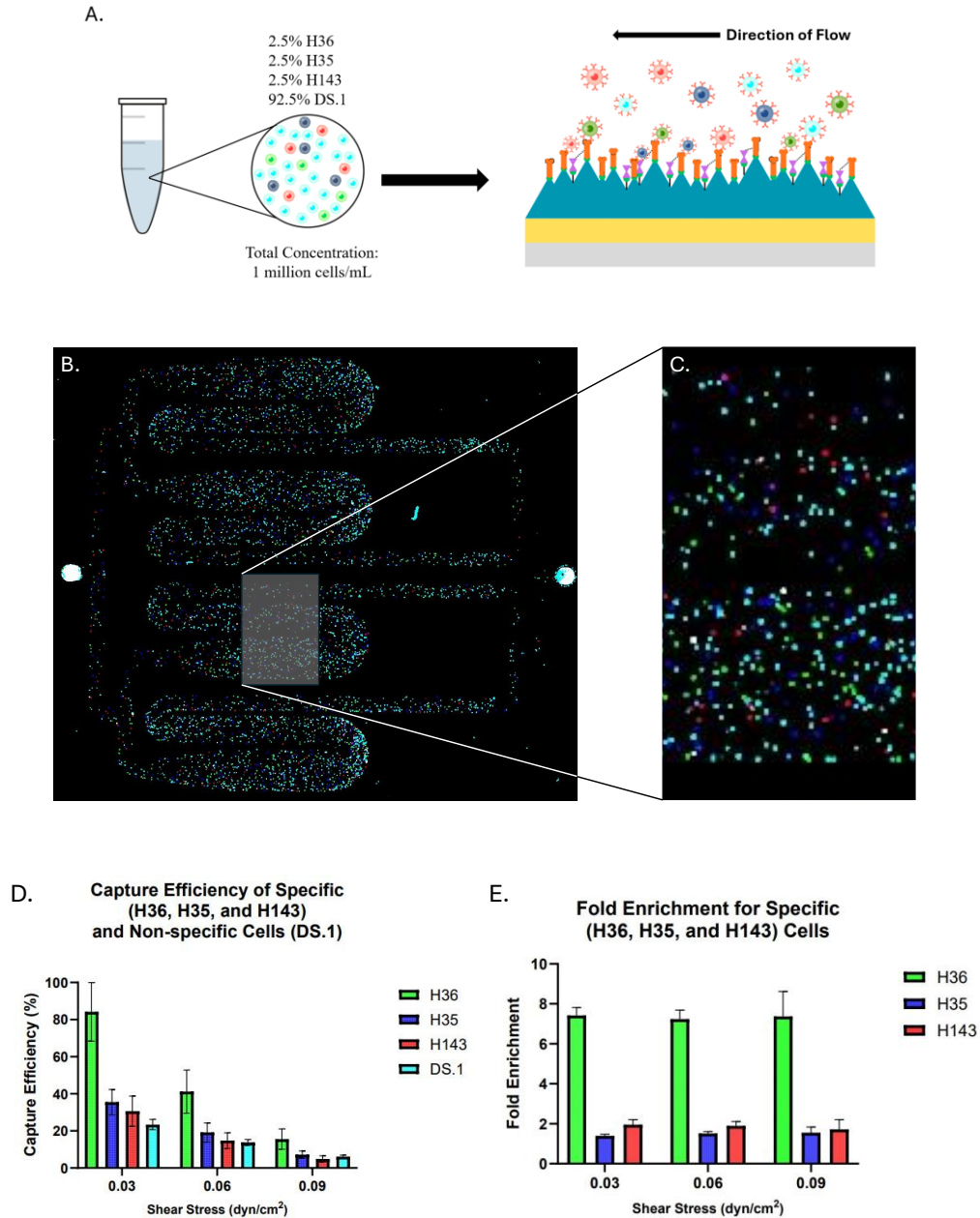


Figure 4.: Schematic of the mixed cell population of hybridoma cell lines and cell flow during perfusion (a). A mixed population of hybridoma cell lines, consisting of 2.5% H36(green), 2.5% H35 (blue), 2.5% H143 (red), and 92.5 percent DS.1 (cyan) were perfused through each channel (b) and adhered to each channel (c). Capture efficiency of the hybridoma cell lines (d) and fold enrichment of specific cells (H36, H35, and H143) (e) were analyzed.

Hybridoma cell lines with defined binding affinities (H36, H35, and H143 in decreasing order of affinity) were used for this experiment²². The DS-1 hybridoma, which does not recognize HA, was used as a negative control. H36, H35, H143, and DS.1 were labelled with CellTrackers CMFDA (green), Deep Red, CMMTR (orange), and BMGC (violet), respectively. These cell lines were then mixed, targeting a final concentration of 1 million cells/mL. To mimic the low percentage of memory B cells, as well as a range of binding avidities, that are present in the peripheral circulation, 7.5 percent of the total concentration consisted of an even distribution between the specific cells (H36, H35, and H143) (**Fig. 4A**). 92.5 percent of the total concentration were DS.1 cells, which are non-specific to Biotin HA. Perfusion occurred for one hour at a flow rate of 80 ul/hr, and washing occurred for 2 hours. Images are acquired using FV1200 Fluoview confocal laser scanning microscope (Olympus) and analyzed using ImageJ (**Fig. 4B,C**).

The videos acquired during perfusion were used to quantify the influx of cells entering the device to obtain an accurate number of cells that entered. The number of cells entered is then used to quantify capture efficiency and fold enrichment. All three hybridoma cell lines show a progressive decrease in capture efficiency as the shear stress increase. The order of capture efficiency is consistent with previous reports: H36>H35>H143. DS.1 cells exhibited the lowest capture efficiency at each shear stress, which is expected as DS.1 cells are non-specific to Biotin HA (**Fig. 4D**). The fold enrichment of each specific cell type remains uniform through the

varying shear stresses. H36 cell maintains the highest fold enrichment, demonstrating enrichment of this cell type in each channel when compared to H35 and H143 (**Fig. 4E**).

4.3. B cells from flu-infected mice can be preferentially enriched

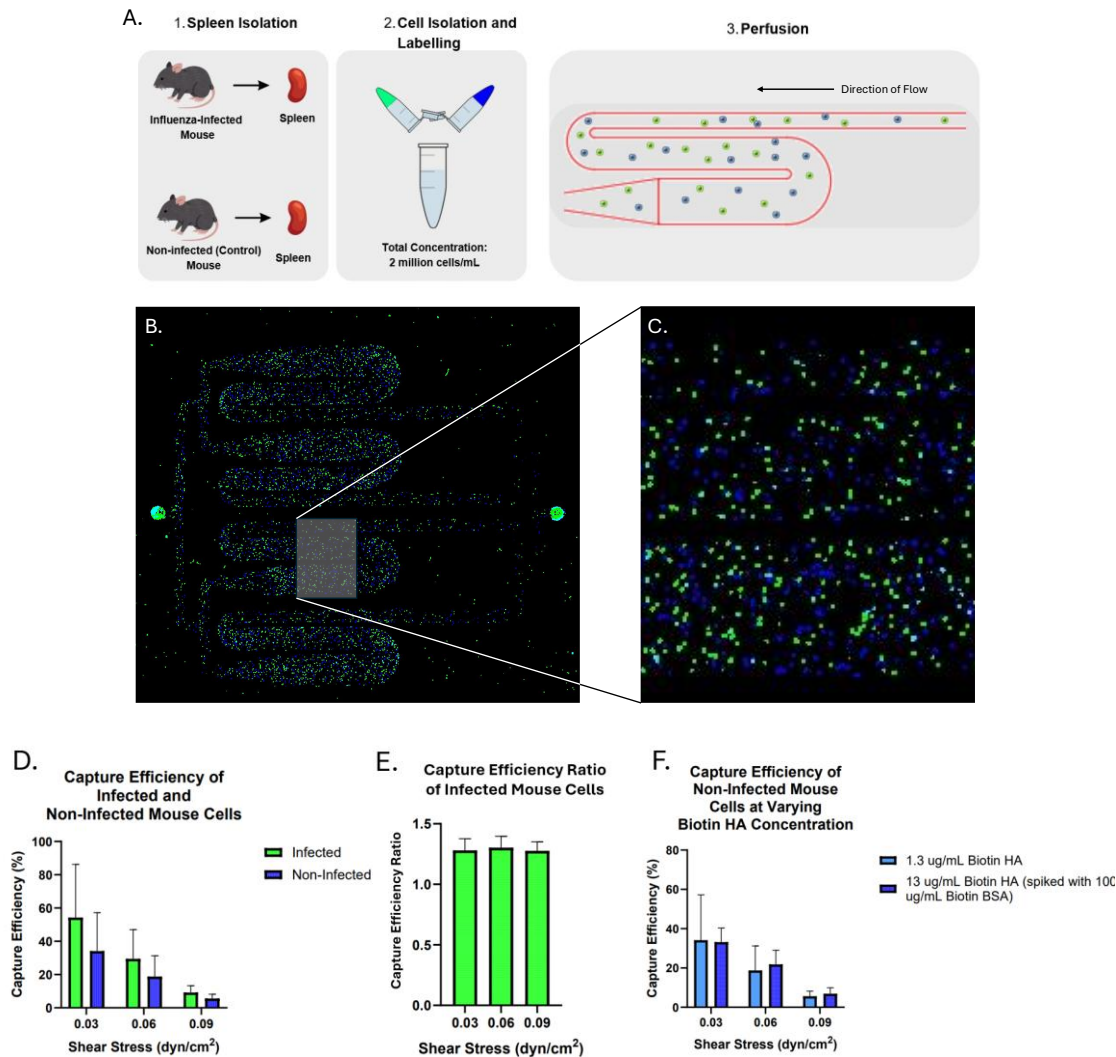


Figure 5: Schematic of the spleen isolation of mouse cells, cell isolation and labeling, and cell perfusion (a). A mixed population of infected and uninfected mouse B cells were perfused through the serpentine device (b) and adhered to each channel (c). Capture efficiency of infected and uninfected mouse cells (d) and capture efficiency ratio of infected mouse cells (e) were analyzed. Further experiment with uninfected occurred with the device coated with 13 ug/mL Biotin HA. The capture efficiencies between uninfected cells based on Biotin HA concentration were compared.

We next tested whether our device could isolate or enrich a population of B cells from a mouse infected with the influenza virus (H1N1). The inlet flow was the same as that was used for separating the hybridoma cell lines. The protocol for coating the devices also remained the same. B cells isolated from the spleen of infected mice cells were labeled with CellTracker CMFDA (green) and uninfected mice cells were labelled with CellTracker Deep Red. After labelling, the infected and uninfected mice were mixed, targeting a 1:1 ratio and suspended at a final concentration of 1.25 million cells/mL (**Fig. 5A**). Perfusion occurred for forty minutes at a flow rate of 80 ul/hr and washing occurred for 80 minutes. Images were acquired using FV1200 Fluoview confocal laser scanning microscope (Olympus) and analyzed using ImageJ (**Fig. 5B,C**).

The inlet of the device showed a greater amount of infected than uninfected mouse B cells adhering in the inlet. Thus, to ensure accurate analysis of cells adhering on the channels, additional analysis was performed in the inlet section of the device to determine the experimental ratio of infected and uninfected cells that actually entered the device. The adhered cells in the inlet were subtracted from the total number of cells added to the inlet to determine the net number of cells that were perfused through the device. Approximately forty-eight percent of infected cells and fifty-two percent of uninfected cells entered the device. This ratio was

confirmed using an additional method of analysis, in which fifteen-second timelapse videos of the cells were acquired prior to entering each device. A particle count was conducted in the perfusate. By this method, approximately forty-seven percent of infected and fifty-three percent of uninfected cells were perfused through the device. The ratio of both methods are similar. However, considering the video used were closer to the device than the inlet, the ratio of 47% of infected and 53% of uninfected mouse cells were used for further analysis for capture efficiency and fold enrichment.

The capture efficiency is greater for infected mouse cells in each shear stress that was tested, with greater difference in capture noted at the lowest shear stress. Uninfected mouse cells maintain a lower capture efficiency at all shear stresses (**Fig. 5D**). The capture efficiency ratio of the infected mouse cells is maintained at approximately 1.3, indicating the success of the serpentine device's ability to increase enrichment of infected mouse at each flow rate (**Fig. 5E**).

A second experiment was conducted using only uninfected mouse cells to attempt to reduce non-specific binding in future experiments. A second device was coating with 13 ug/mL of Biotin HA spiked with 100 ug/mL of Biotin BSA. The protocol up to, and including, maleimide activated neutravidin coating remained the same. When comparing the capture efficiencies of uninfected mouse cells with two different concentrations of Biotin HA, it was determined that increasing the concentration of Biotin HA from 1.3 to 13 ug/mL had little effect on the capture efficiency (**Fig. 5F**).

DISCUSSION

The objective of this project was to utilize a microfluidic device with variable serial shear stress in a serial design that can separate (or enrich) and isolate low-avidity memory B cell

populations. Current immunological techniques are limited in forming a comprehensive analysis of our immune responses as the most common methods (e.g., flow cytometry) rely on studying antibodies or equilibrium-based binding of BCR/antibody with antigen, thus neglecting low avidity memory B cells^{21,22,28}. Since low-avidity memory B cells can offer protection when viral mutation occurs due to their cross-reactive interactions, it is essential to develop methods of detecting and analyzing low-avidity memory B cell populations^{7,8,12,13}. The results of this study demonstrate significant potential in the ability to separate and isolate memory B cell population with minimal interference from non-specific cells, thus supporting the use of microfluidic technology for a more comprehensive analysis of our immune response.

To assess the microfluidic technology's ability to separate mixed populations of memory B cells, it was essential that the serpentine device was uniformly coated with antigen (in our case, biotinylated HA). To determine the necessary protocol to ensure even coating, FITC Biotin was coated on each device under three conditions. In the first condition, a device was coated with 13 ug/mL of FITC Biotin. Results showed a high intensity concentration within the first channel, and thus uneven coating throughout the device, as the third channel remained uncoated at the ends of the four hours. In the second condition, another device was coated with 13 ug/mL of FITC Biotin spiked with 100 ug/mL of Biotin BSA – in other words, a total concentration of 113 ug/mL biotinylated protein. Results showed uniform distribution of the fluorescent signal consistent with uniform coating of FITC, as Biotin BSA binds to exposed hydrophobic regions, allowing for even distribution of FITC Biotin. In a third condition, the third device was coated with 1.3 ug/mL of FITC Biotin spiked with 100 ug/mL of Biotin BSA. While there was a more even distribution of FITC Biotin when compared to the first condition, the reduced concentration FITC Biotin resulted in an inability of the third channel being fully coated. Based on the

experiment it was determined that a Biotin HA concentration of 13 ug/mL spiked with 100 ug/mL of Biotin BSA was the best solution for ensuring uniformed coating.

The perfusion of the hybridoma cell lines demonstrated visible separation of three different hybridoma cell lines with known variable binding avidity to HA. The hybridoma cell lines used were H36, H35, and H143, in order of descending binding avidities. DS.1 cells are hybridomas that do not recognize Biotin HA, and therefore, was used as a negative control. Results were consistent with H36 binding significantly in each channel, with lower capture efficiencies of H35 and H143 cells. Fold enrichment of the specific cell lines remained stable at each shear stress. DS.1 cells demonstrated the lowest binding avidity. It was expected that the fold enrichment of H36 cells would be highest at the highest shear, and drastically reduce when entering the lower shears, while H35 and H143 cells increase in enrichment at the lower shear stress. In addition, it was expected that the capture efficiency of H36 cells will remain high in the highest shear stress while plateauing at lower shears. The results of this experiment indicate that while there was success in separating specific cells from DS.1 cells, the shear stresses used in this experiment were too high for each cell type to remain adhered to the device at each shear stress. A lower range of shear stress may allow for the cells to properly adhere to the device as the force from fluid flow across the device may be lower than the binding avidity between the BCR and Biotin HA^{29,30}.

Using the results of the hybridoma experiments, the murine experiment was conducted to demonstrate the translational capability of the serpentine device to enrich mammalian B cells following a flu infection. The cells were isolated from the mouse spleen, and the binding avidity of the mixed B cell population was unknown. A mixed population of influenza-infected mouse cells and negative control uninfected mouse cells were perfused across the serpentine device.

The same protocol was utilized, apart from the Biotin HA concentration, which was 1.3 ug/mL. Results showed that the capture efficiency was higher for infected cells (approximately 52%, 30%, and 10%) compared to uninfected cells (approximately 32%, 18%, and 7%) at all shear stresses, respectively (0.03, 0.06, and 0.09 dynes/cm²). Previous reports using flow cytometry suggest less than 0.5% of the cells isolated from the spleen following flu infection in the mouse are HA-specific^{21,31}. Therefore, while the difference in capture efficiency may appear subtle, it is highly significant as one can use this difference to calculate a minimum fraction of the mouse B cells that are HA-specific in the infected mouse. The total number of cells captured from the B cells isolated from the infected mouse is simply the sum of the HA-specific cells and the non-specific cells, or

$$C_{cap} = C_{tot}E_{s,n} = C_{tot}F_sE_s + C_{tot}F_nE_n$$

Where C_{tot} and C_{cap} are the total cells that enter the device and total cells adhered, respectively; $E_{s,n}$, E_s , and E_n are the capture efficiency of specific and non-specific cells together (i.e., the infected mouse), the specific cells alone, and the non-specific cells alone, respectively; and F_s and F_n are the fraction of specific and non-specific cells, respectively. Recognizing in this binary system that $F_n=1-F_s$, one can quickly solve for F_s to get,

$$F_s = \frac{E_{s,n} - E_n}{E_s - E_n}$$

$E_{s,n}$ and E_n are directly measured in our experiment. One can then see that a *minimum* value of F_s is calculated if we assume that all HA-specific cells are captured, or $E_s=1$. This assumption is most likely to be valid at the lowest shear (0.03 dynes/cm²); thus, under this condition $F_s=0.29$, or a minimum of 29% of the cells from the infected mouse could have HA-specificity. While

this number represents only an estimate, note that it is much larger than the estimate using flow cytometry (0.5%) and it also represents a minimum value.

As shear rate is lowered, the number of cells binding from the uninfected mouse should eventually equal the number of cells binding from the infected mouse as non-specific binding dominates. However, we observe that the ratio of the capture efficiency of the cells from the uninfected and infected mice is constant. Therefore, lowering the shear rate below 0.03 may recover the lowest avidity HA-specific cells.

These results suggest that the serpentine device can detect and enrich relevant cell populations from a mixed cell population. Additional experiments with the uninfected mouse were conducted with a device coated with 13 ug/mL Biotin HA spiked with Biotin BSA in an attempt to further reduce nonspecific binding. Results showed negligible improvements in reducing nonspecific binding. It is reasonable to assume that if the mixed population of infected and non-infected mouse cells were perfused at a higher Biotin HA concentration, there will be greater capture efficiency and enrichment of infected mouse cells. Although increasing the shear stress to minimize non-specific binding will increase fold enrichment, this is at a cost of losing the lowest avidity HA-specific cells³².

While the serpentine device was successful in limiting nonspecific binding, there are still unanswered questions and improvement that could be made. First, the serpentine device was designed to only test three shear stresses at a time. Some cells that did not adhere during perfusion could have potentially adhered to lower shear stresses. With the hybridoma cell lines, the fold enrichment that was analyzed was not consistent with our expectations, indicating range of the shear stress was too high to achieve selective enrichment of the populations in the different shear regions. The second limitation is regarding the mouse cells and our inability to sort out the

mixed B cell population present. Unlike the hybridoma cell lines, which were characterized prior to perfusion, there is no gold-standard by which to characterize the mouse cells into HA-specific and non-specific.

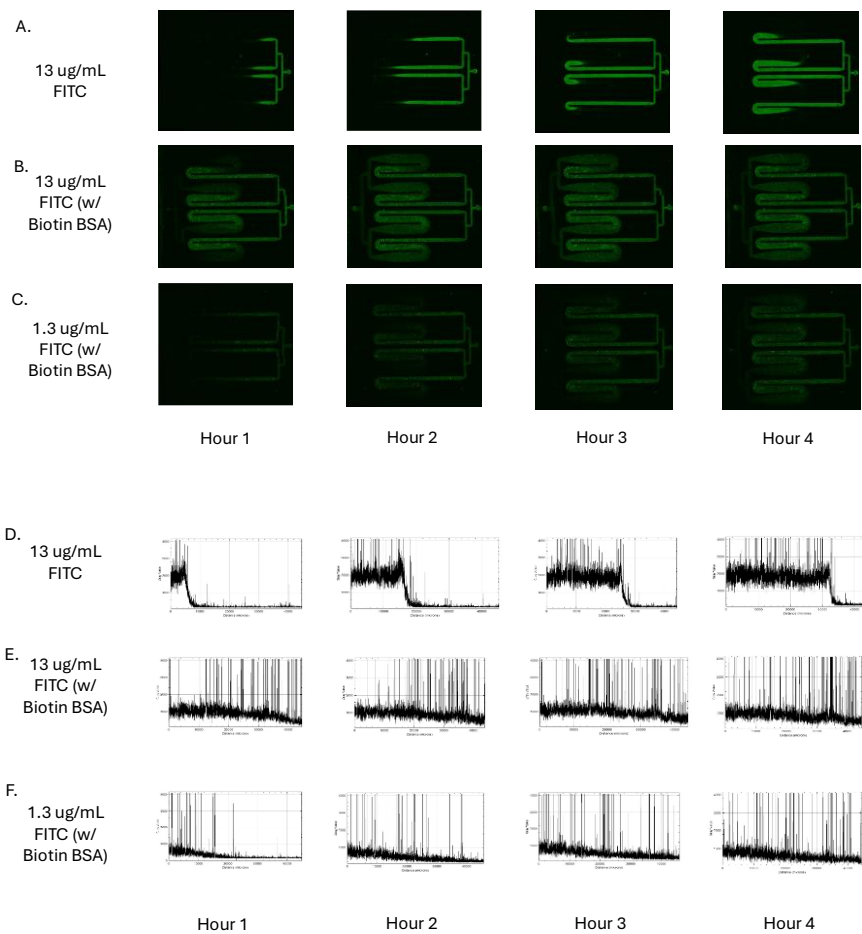
Future work includes the ability to release the hybridoma cell lines and mouse cells from the device for further characterization (e.g. measure the avidity of the antibodies produced from the captured B cells). The current serpentine device is unable to release the cells from the individual cell lines without preventing the higher-avidity cell lines from mixing with the lower-avidity and nonspecific cells. Finally, additional channels could be added to the serpentine device to study the behavior of B cells and their ability to attach in a wide range of shear stress. This will allow for further enrichment of hybridoma cell lines and infected mouse cells, and lead to a more comprehensive analysis of our adaptive immune system.

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SUPPLEMENTAL FIGURES



Supplementary Figure 1: Images of FITC Biotin coating when device were coated with 13 ug/mL FITC Biotin (a), 13 ug/mL FITC Biotin spiked with 100 ug/mL Biotin BSA (b), and 13 ug/mL FITC Biotin spiked with 100 ug/mL Biotin BSA (c). Profile plots were generated using ImageJ to indicate the intensity of FITC Biotin through each channel (d, e, f).