A MICROFLUIDIC PLATFORM FOR SCREENING AND SELECTION OF MONOCLONAL ANTIBODIES FROM SINGLE CELLS

Anupam Singhal¹,²,³,⁴*, Daniel DaCosta²,⁴, Charles Haynes¹,², and Carl Hansen²,⁴

¹ Department of Chemical and Biological Engineering, ²Department of Physics and Astronomy, ³Michael Smith Laboratories, ⁴Centre for High-Throughput Biology, University of British Columbia, Canada

ABSTRACT

We report a microfluidic platform for rapid screening and selection of monoclonal antibodies (mAbs) secreted by single cells. The microfluidic platform integrates single-cell handling and compartmentalization, fluorescence detection of antibodies secreted by cells, and cell recovery for reverse transcription polymerase chain reaction (RT-PCR) of antibody genes.

KEYWORDS: Microfluidics, Single-cell analysis, Antibodies, Monoclonal Antibodies, Binding kinetics, RT-PCR

INTRODUCTION

Antibodies are defense proteins produced by the vertebrate adaptive immune system for the purposes of binding and targeting a diverse range of bacteria, viruses, and other foreign molecules (antigens). As a result of their ability to bind target antigens selectively and with high affinity, monoclonal antibodies (mAbs) are invaluable tools for protein purification, cell sorting, and diagnostics. In addition, mAbs are the most rapidly growing class of therapeutics and the second largest group of drugs after vaccines, with 21 products approved for clinical use and over 150 others in development for the treatment of cancer, cardiovascular diseases, autoimmune disorders, and infectious diseases.[1]

Conventional mAb production involves the immunization of animals (i.e. mice) with a target antigen, such as a virus, bacteria, or foreign protein. The immunized produce a produce on the order of $10^5$ antibody-secreting cells (ASCs), each with the capacity to produce a unique mAb specific to the target antigen.[2] Single ASCs do not produce antibodies in sufficiently large quantities (i.e. micrograms) for affinity measurement using standard techniques; thus, each ASC must be clonally expanded in order to select which cells are producing antibodies of desired affinity and selectivity to the target antigen. As primary ASCs cannot be efficiently grown in laboratory tissue cultures, clonal expansion is achieved by fusing ASCs to murine myeloma (cancer) cells to produce immortalized, antibody-secreting (hybridoma) cells. Expansion of each successfully created hybridomas then produces a mAb in sufficiently high concentrations to measure its affinity and selectivity to a target antigen. A long-recognized limitation of hybridoma technology is the low efficiency of the fusion process; that is, whereas an immune response may produce on the order of $10^{-4}$ antibody-secreting cells, a typical fusion will yield less than 100 viable hybridomas. Therefore, fusions from tens to hundreds of animals are required to fully sample the antibody diversity produced in an immune response, making the hybridoma approach both time-consuming and expensive.

As every antibody-producing cell (ASC) in the vertebrate immune system can produce a unique antibody, ASCs are an ideal system for single-cell analysis. Recently, antibodies have been selected from single cells without clonal expansion.[3] ASCs are harvested from immunized animals and monoclonal antibodies are produced by single-cell reverse-transcription polymerase chain reaction (RT-PCR) of antibody genes, followed by cloning and expression of these genes. Subsequently, all antibody genes are cloned and expressed in order to select antigen-specific mAbs, thereby making these single-cell techniques similarly expensive and time-consuming to other approaches requiring clonal expansion. We hypothesized that, by compartmentalizing single cells in micro-fabricated wells $10^3$ times smaller in volume (< 1nL) than conventional cell culture wells (>1μL), we could screen and select antibodies produced by single cells for their affinity and selectivity to target antigens. As ASCs are known to produce antibodies at a very high rate (1000s of molecules per second), they secrete detectable concentrations (>1nM) of antibodies within hours of incubation in a sub-nanoliter chamber. In contrast, in conventional 96-well plates, primary ASCs need to be cultured for up to one week in order to obtain detectable antibody concentrations; however, these cells cannot be efficiently cultured in laboratory conditions for longer than 1 day. Previous studies have shown that antibodies secreted by single cells can be detected in microfabricated sub-nanoliter wells using fluorescence microscopy. [4-5] We have developed a novel microfluidic technology for rapid screening and selection of monoclonal antibodies from single antibody-secreting cells without clonal expansion. The microfluidic platform integrates the handling and compartmentalization of single cells, fluorescence measurements of antigen binding kinetics of antibodies secreted by single cells, and recovery of selected cells for off-chip reverse transcription polymerase chain reaction (RT-PCR) of antibody genes.

THEORY

The affinity (“binding strength”) of an antibody for the target antigen is a critical parameter when an antibody is selected for a given application. Although the affinity of an antibody-antigen interaction is typically quantified by an equilibrium dis...
association constant (K_d), which describes the dynamic equilibrium between binding and unbinding events, the kinetic association and dissociation rate constants (k_{on} and k_{off}) provide a more complete characterization of an antibody-antigen interaction. The equilibrium and kinetic rate constants for an antibody-antigen interaction are defined by the following first-order mass action and Langmuir isotherm equations:

$$[AbAg] = [Ab]_0 \frac{[Ag]}{[Ag]_0 + K_d \left(1 - e^{-t(k_{on}[Ag]_0 + k_{off}t)}\right)}$$  \quad (1)

$$[AbAg] = [Ab]_0 \frac{[Ag]}{[Ag]_0 + K_d \left(1 - e^{-k_{off}t}\right)}$$  \quad (2)

$$K_d = \frac{k_{off}}{k_{on}}$$  \quad (3),

where [AbAg], [Ab]_0, [Ag]_0 are the concentrations of antibody-antigen complex, antibody, and antigen respectively.

**EXPERIMENTAL**

We designed and fabricated PDMS microfluidic devices consisting of over 100 individually-addressable chambers using multilayer soft lithography. In each chamber, a single ASC is incubated with antibody-capture beads for 1 hour, after which, all chambers are flushed with fluorescently labeled antigen and fluorescence microscopy is performed to detect antigen-specific monoclonal antibodies (mAbs) produced by single ASCs. Subsequently, single ASCs secreting antigen-specific mAbs are recovered from the device to perform RT-PCR on the heavy and light chain genes encoding antibodies of interest. We then directly measure antibody-antigen binding kinetics by time-course fluorescence microscopy of antibody-captured beads immobilized in microfluidic traps and subject to a series of wash cycles with fluorescently labeled antigen and buffer (Figure 1).

**RESULTS AND DISCUSSION**

As a proof of concept, we have demonstrated the screening and selection of antibodies to the model antigen hen egg lysozyme (HEL) from single hybridoma cells (Figure 2). Anti-lysozyme monoclonal antibodies produced by two different hybridoma cells (D1.3 and HyHEL-5) were discriminated on the basis of their different association rate, dissociation rate, and equilibrium dissociation constants. Furthermore, we have demonstrated that we can selectively recover antibody-secreting cells from the device and perform off-chip RT-PCR of the heavy and light chain genes from these cells (Figure 3). At present, we are extending this work to screen splenocytes harvested and purified from mice immunized with hen egg lysozyme, select novel anti-lysozyme monoclonal antibodies (mAbs), and amplify and sequence the heavy and light chain genes encoding these mAbs.

**CONCLUSION**

We envision that this platform will be useful for rapid and inexpensive selection of monoclonal antibodies to a diverse range of antigens from many different species, including mice, rabbits, and humans subject to vaccination or naturally-occurring infections.
Figure 2: (Left) Brightfield microscope image of a single hybridoma cell adjacent to antibody capture beads trapped using a microfluidic sieve valve. (Right) Antibody-antigen binding kinetics measured from antibodies secreted by a single D1.3 hybridoma cell using the fluorescence bead assay. Figure reproduced from reference [6].

Figure 3: 1% DNA agarose gel to visualize products from single-cell RT-PCR of heavy and light chain antibody genes. RT-PCR performed on single ASCs secreting anti-lysozyme monoclonal antibodies.

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CONTACT
*Anupam Singhal, tel: +1-604-8273547; asinghal@chbe.ubc.ca