MICROFLUIDIC SELECTION OF LIBRARY ELEMENTS

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ABSTRACT

We demonstrate that biological libraries can be screened using microfluidics. A bacteriophage library encoding dodecapeptides is passed through a microfluidic chip wherein antibodies are immobilized. Bacteriophages exhibiting affinity for the antibody are retained and eluted in a subsequent step. The high degree of control over flow and binding conditions during the screening enables dramatic reduction of the native library in favor of bacteriophages binding to the antibody target.

KEYWORDS: library screening, bacteriophage, microfluidics

INTRODUCTION

Biological libraries provide extraordinary (bio)chemical diversity. Yet, exploiting this diversity requires identifying elements of interest, such as ligands for specific receptors, from libraries that can have up to 10^{11} different elements per µL. Bacteriophage libraries can for example be screened for discovering specific antibodies to viral receptors and peptidic ligands for nanostructures [1, 2]. Screening libraries uses conventional microtiter plates with the exception of very few examples wherein library elements are incubated with receptors off-chip and sorted on-chip [3]. This paper reports a method for selecting library elements within a microfluidic chip.

EXPERIMENTAL

M13 phages bearing a potential ligand for a surface-immobilized receptor (the target) are screened under "microfluidic conditions" and after washing and elution, the phages are recovered and analyzed off-chip using conventional biotechnology techniques, Fig. 1. Protein targets are deposited from solution onto a poly(dimethylsiloxane) (PDMS) layer that is used to seal a microfluidic chip.

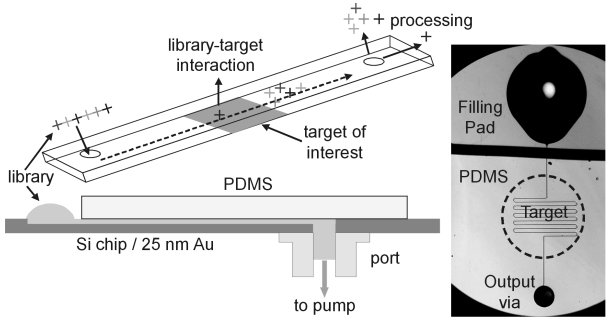

Figure 1: Sketch (left) and micrograph (right) of a microfluidic chip sealed with a PDMS layer for screening biological libraries.

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RESULTS AND DISCUSSION

Screening under microfluidic conditions refers to the possibility of analyzing as little as 1 µL of library solution, using small receptor areas, and employing precise volumes for rinsing and elution steps. Control over volumes and composition of solutions and step durations may help efficiently reduce the size of the library at each screening round.

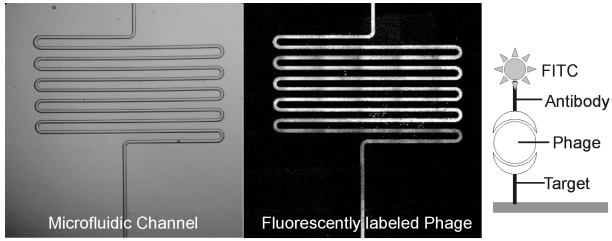

Figure 2: A target immobilized on a sealing PDMS surface is exposed to a library flowing in a meandering microfluidic channel (20 µm deep and 60 µm wide, typical flow rate of 10−20 nL/s). Using a monoclonal antibody target directed against a coat protein of the phage, a fluorescence immunoassay was effected to demonstrate that phages in solution bind antibodies in a microfluidic device.

Figure 2 shows phages bound to a receptor on PDMS that are detected using fluorescently-labeled antibodies. In the example shown in Figure 3, a dodecapeptide library was screened against streptavidin. In only one screening round, tittering the input library, the eluate, and the solution used to wash non-specific binders revealed a reduction of the native library from ~10^{11} phages to ~10^{3} phages in 10 µL. The fraction of the library that was washed had ~10^{8} phages.

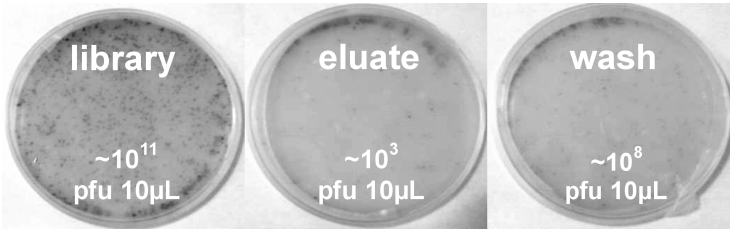

Figure 3: A phage library encoding dodecapeptides is screened against a streptavidin target using a microfluidic as shown in Fig. 1. The library complexity is strongly reduced in only one round. The sizes of the initial library, non-interacting fraction of the library ("waste"), and fraction having affinity for the target (eluate) are titered off-chip using conventional biotechnology protocols.

Screening libraries encoding hemagglutinin variants against antibodies may give insight on the evolutionary competition between anti-hemagglutinin antibodies and mutating hemagglutinin proteins [1]. As this may result in understanding better how hemagglutinin binds cell receptors from different species while escaping immune responses, we screened a hemagglutinin epitope library against an antibody known to

bind the YPYDVPDYA sequence of influenza viruses. Figure 4 shows 2 sequences corresponding to phages recovered after one screening round. A clear selection of phages having affinity for anti-hemagglutinin antibody occurred.

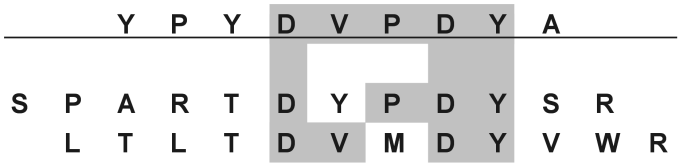

Figure 4. Example of two sequences obtained in one round of screening using a dodecapeptide library and an antibody against a hemagglutinin epitope composed of nine amino acids (library and antibody were from USBiological).

CONCLUSIONS

While this work explores a proof-of-concept for screening biologically diverse libraries using microfluidics, its concept is general. Many types of libraries can in principle be screened using microfluidic chips as long as targets can be conveniently immobilized on PDMS or in some specific areas of the chip itself. We note that the possibility of patterning targets on PDMS and splitting microfluidic flow paths [4] open a new dimension for screening libraries wherein "logical" screening can be performed. Specific library elements may not be selected only based on binding criteria but can also be selected based on non-binding criteria.

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