Peptide Nucleic Acid-based Microfluidic Chip for Isothermal HIV Detection

Christopher Yin, Nicole Morgan, Tom Pohida, Kaylyn Oshaben, Daniel H. Appella†

†*Laboratory of Bioorganic Chemistry, NIDDK, Bethesda, Maryland 20892*

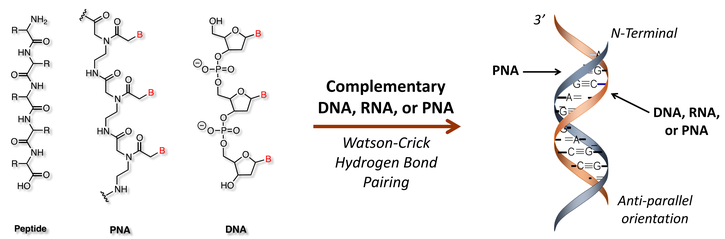
National Institute of Diabetes and Digestive and Kidney Diseases

Abstract

Regular monitoring of an HIV patient's viral load - the amount of free virus in the bloodstream - is crucial for clinical interventions that can maintain immunological integrity and prevent the spread of drug-resistant HIV. However, the current standards for viral load testing are PCR-based and require expensive equipment not suitable for low-resource settings, where the burden of HIV is highest. Remote, centralized testing facilities limit the financial burden on local clinics but greatly increase the time-to-result, reducing the test's clinical utility and hindering patient retention. Here, we report on the development of a point-of-care viral load test incorporating an isothermal, PNA-based amplification method into a microfluidic device.

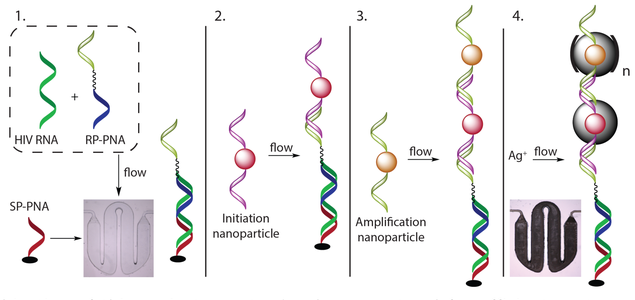
Peptide Nucleic Acid

Peptide nucleic acids (PNAs) are synthetic homologs of nucleic acids in which the phosphate-sugar backbone is replaced by a pseudo-peptide oligomer. PNA forms stable duplexes with complementary DNA, RNA, or PNA according to Watson-Crick base pairing. PNA-RNA duplexes are stronger than DNA-RNA.



Device Overview

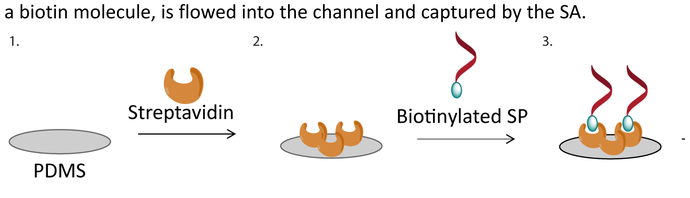
Our device employs a sandwich assay that detects a conserved HIV RNA sequence and amplifies the signal through a series of PNA coated gold nanoparticles. A PNA surface probe (SP) immobilized in the PDMS microchannel captures the target sequence, which has been tagged by a PNA reporter probe (RP) (1). PNA-conjugated gold nanoparticles bind to the RP through complementary PNA interactions (2). A second PNA-conjugated nanoparticle binds to the initiation nanoparticle, forming gold clusters around the captured target sequence (3). Signal amplification is achieved by adding silver enhancer solution to the channel providing a visual readout (4).



The objective of this project was to develop a protocol for efficient capture of the surface probe and sandwich complex in the channel through optimization of wash times, flow rates, and blocking steps.

Channel Preparation

To capture the SP in the PDMS channel, a layer of streptavidin (SA) is first immobilized to the channel surface. The strong hydrophobicity of PDMS allows for the ready adsorption of uncharged proteins. Second, the surface probe PNA, functionalized with a biotin molecule, is flowed into the channel and captured by the SA.



Streptavidin Immobilization

Maximum streptavidin surface density is desired to increase the assay sensitivity. The concentration of SA incubated in the channel was varied, and no significant increase in SA coverage was observed above a concentration of 5 µM.

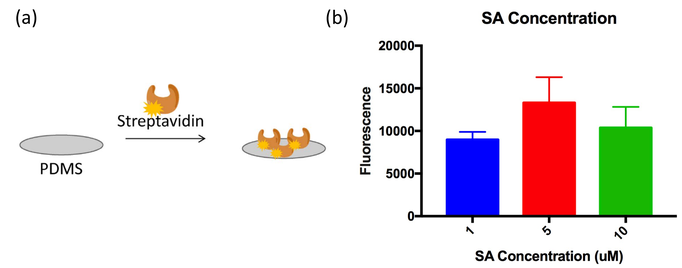


Figure 1. *(a) Fluorophore-labeled SA is incubated in the channel at differing concentrations. (b) The channel is saturated above 5 µM.*

It is imperative that any weakly bound streptavidin is washed from the channel after the immobilization step to prevent loss of signal. It was determined that a 2 minute wash step with PBS at a flow rate of 1 mL/min was sufficient for the streptavidin coverage of the channel to stabilize and remain constant over further washing. The flow rate of the wash step was also examined to ensure SA is not stripped from the channel during the wash step. A flow rate of 1mL/min was observed to have no appreciable difference in SA removal from a rate of 0.1mL/min.

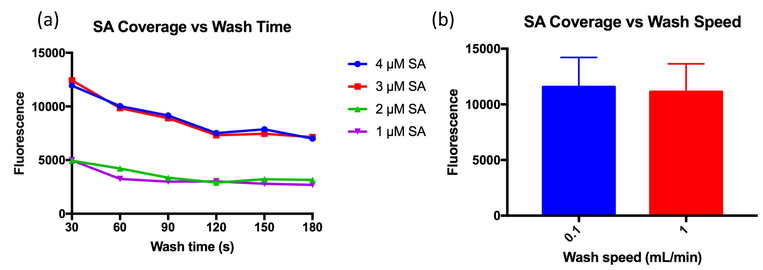


Figure 2*. (a) Fluorophore-labeled SA is incubated in the channel before being washed with PBS in consecutive 30s intervals at a flowrate of 1mL/min. Channels incubated in different concentrations of SA exhibit the same trend. (b) Fluorophore-labeled SA is incubated in the channel and washed at a flowrate of either 0.1 mL/min or 1 mL/min. No significant difference in SA retention is observed.*

BSA Blocking

Both the accuracy and sensitivity of the assay are dependent upon sequence-specific interactions, and it is necessary to minimize non-specific interactions within the channel. BSA is a small globular protein commonly used to reduce nonspecific interactions. A 1% BSA blocking solution was found to minimize the surface of the PDMS channel not covered by the SA layer.

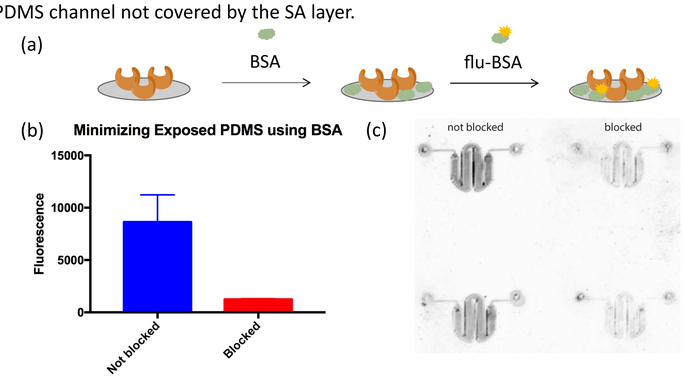


Figure 3*. (a) Channels are incubated with SA, then either PBS or 1% BSA. Fluorophore-labeled BSA (flu-BSA) is incubated in each channel before washing. (b) Blocking is shown to significantly reduce further protein immobilization to channel surface. (c) Corresponding image from gel reader.*

Surface Probe Capture

It was desired to saturate the channel with surface probe while avoiding material waste. The concentration of fluorescein-labeled biotin (flu-biotin) incubated in the SA-coated channel was varied, and no significant increase in flu-biotin capture was observed above a concentration of 1 µM.

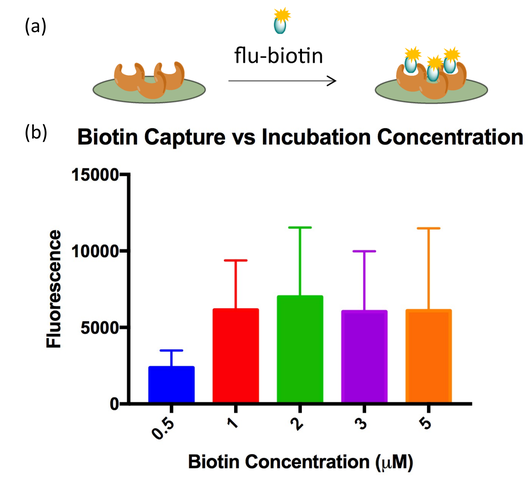


Figure 4*. (a) Channels are coated with SA and blocked with 1% BSA in PBS, before being incubated with flu-biotin. (b) A concentration of 1 µM flu-biotin appears sufficient to saturate the channel.*

The effects of blocking were explored by observing the capture of flu-biotin as a surrogate for the surface probe on the SA-coated channel. Blocking was shown to have minimal impact on biotin retention, indicating that capture of the surface probe can occur before or after the blocking step. The surrogate surface probe showed minimal interaction with the PDMS channel.

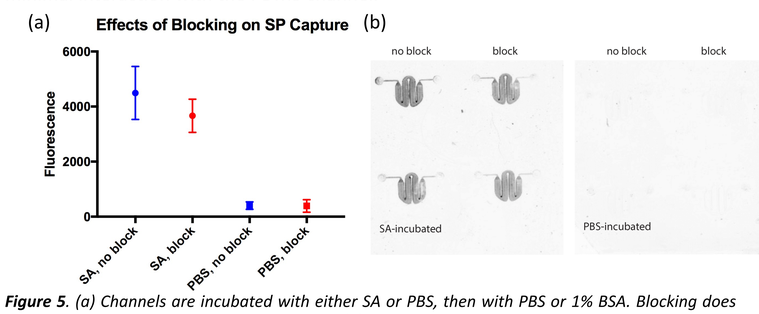
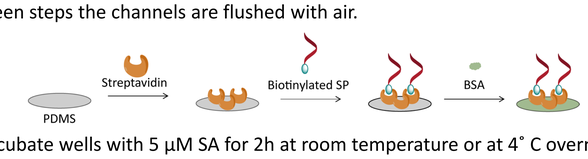


Figure 5*. (a) Channels are incubated with either SA or PBS, then with PBS or 1% BSA. Blocking does not have a significant effect on biotin capture. (b) Corresponding image from gel reader.*

Optimized Protocol

An optimized protocol for preparation of the microfluidic channel is reported below. In between steps the channels are flushed with air.



1. Incubate wells with 5 µM SA for 2h at room temperature or at 4˚ C overnight.
2. Wash with PBS, 1 mL/min for 2 min.
3. Incubate 1 µM surface probe (SP) for 10 min at room temperature.
4. Wash with PBS, 1 mL/min for 2 min.
5. Incubate wells with 1% BSA in PBS for 1h at room temperature.

References

1. Zhao, C., Hoppe, T., Setty, M.K.H.G., Murray, D., Chun, T.W., Hewlett, I., Appella, D.H. Quantification of plasma HIV RNA using chemically engineered peptide nucleic acids. *Nature Communications* 5, 5079 (2014).
2. "HIV/AIDS: Data and Statistics." *World Health Organization*, World Health Organization, www.who.int/hiv/data/en/.
3. "Viral Load Testing Capacity Still Weak in Sub-Saharan Africa, 7-Country Study Finds." *HIV & AIDS Information*, www.aidsmap.com/Viral-load-testing-capacity-still-weak-in-sub-Saharan-Africa-7-country-study-finds/page/3107907/.