Abstract

One of the many great challenges that medical diagnostics face is the need for sensitive, reliable, and rapid detection of molecules in very complex solutions such as blood or urine. DNA-based biosensors have shown great promise in terms of sensitivity and reliability for target detection, but the need for rapid testing has considerably slowed their use in practical applications within the medical world. In the research to be conducted, we explore the incorporation of DNA-based biosensor into a lateral flow assay format (similar to the common at-home pregnancy test for human chorionic gonadotropin in urine). To facilitate this, we are developing a gold nanoparticle decorated with a functional DNA probe that recognizes and binds to botulism neurotoxin variant A (BoNTA). This conjugate then wicks across a nitrocellulose membrane to specific capture points, allowing rapid visual assessment of the BoNTA contamination of a sample. In the future, we aim to demonstrate that this represents a generic platform for detection that could be used with any existing DNA aptamer-based biosensing technique and can be applied to many medical settings, including small clinics, without the need for technicians to operate the biosensor.

Background

Lateral Flow Assays (LFAs) are rapid assay methods that allow for visual confirmation of a desired target. LFAs are also known as immunochromatography assays, aptamer chromatography assays, dipsicks, and strip tests. LFAs allow for a fast preliminary diagnosis of a wide variety of analyte. One of the most commonly used LFAs are household pregnancy tests. These assays specifically test for the presence of human chorionic gonadotropin in a potential mother’s urine. Aside from being used in household products, LFAs are also used in clinical, veterinary, agricultural, food industry, biodefence, and environmental applications. The range of different molecules that a LFA can detect within these applications is quite extensive. A few examples of these molecules include...

Gold Nanoparticle (AuNP) Properties

A main component of a lateral flow assay is gold nanoparticles (AuNPs). These particles have been extensively used in biological and technological applications because of their unique optical properties. Because these gold particles are smaller than visible light, they can be used to create a solution in our lateral flow assay, this allows for the visualization of the test and control line in the form of a red/purple line depending on the molecules present in the analyte. The size and concentration of the AuNPs are optimized to allow for the visualization of the test and control line.

Gold Colloid Plasmon Resonance

Figure 1. (Left) Oligonucleotide showing color changing corresponding to concentrations of antigen in distilled water. (Right) Assembly of colloidal gold-based dipstick.

Various techniques exist that offer high rates of detection, including PCR, cytometry, or cell enrichment. However, these are time-consuming, expensive, and may require target enrichment or production of other biomolecules (i.e. antibodies). LFAs serve as a highly sensitive, rapid, and inexpensive tool which can help provide a solution to these problems. However, many LFAs so far have used antibodies in their construction and have a relatively long assay time or multiple washing and separation steps. DNA-aptamer based LFAs have high specificity, low molecular weight, easy and reproducible production, versatility in application, easy discovery and manipulation, and eliminate the need for washing and/or waiting for an extended amount of time.

DNA Aptamer based versus Antibody Based LFAs

Lateral Flow Assays

Figure 2. (Left) Schematic of localized surface plasmon resonance (LSPR) where the free conduction electrons in the metal nanoparticles are driven into oscillation due to strong coupling with incident light. (Middle) Solution of gold nanoparticles of various sizes. The color difference causes the difference in colors. (Right) Bonham lab-produced AuNPs.

Lateral Flow Assays (LFAs) are rapid assay methods that allow for visual assessment of the BoNTA contamination of a sample. In the future, we hope to incorporate Botulism toxin into one of our LFA designs. To further test the limits of our assays, we intend to conduct tests with pure Botulism toxin and contaminated cows blood.

References


Bonham Laboratory Website: http://BonhamLab.com

Figure 3. Comparison of antibody and aptamer AuNP.

Future Directions

Currently we have been constructing LFAs that are sensitive to DNA from an E. Coli strain. Optimization of the test and control line visibility/definition as well as the optimum buffer amount are being tested. In time, we hope to incorporate Botulism toxin into one of our LFA designs. To further test the limits of our assays, we intend to conduct tests with pure Botulism toxin and contaminated cows blood.

Acknowledgements

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Development of a Botulism Neurotoxin Sensitive Lateral Flow Assay Biosensor for Clinical Applications and Medical Settings

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Figure 5. (Left) Oligonucleotide showing color changing corresponding to concentrations of antigen in distilled water. (Right) Assembly of colloidal gold-based dipstick.

Figure 6. (Left) Schematic demonstrating the method of a antibody based LFA. (Right) A schematic of the assay steps and results.

Assuming the presence of the target DNA in your analyte, the LFA works in just a few steps:

1. Desired analyte added to the sample pad
2. Analyte travels across conjugate pad and target DNA will interact with the AuNP-
   DNA probe conjugate
3. Target DNA bound AuNP-DNA conjugate travels across the nitrocellulose membrane
   and interacts with the DNA probe test line. The AuNP conjugate will stick and a red line will be visible
4. Any unbound AuNP-DNA probe conjugates will flow past the test line and interact
   with the control line to give second visible red line

Bonham Lab Constructed Lateral Flow Assays

Figure 7 (Left) Test lab constructed LFA with penty to convey dimensions. (Right) Lab constructed LFA with AuNP-DNA flowing across nitrocellulose to control line.

Aptamer Discovery for Novel Detection

Research in the Bonham lab has revolved around developing biosensors which can be used for a variety of targets. Targets that have a biosensor already created are...

• breast cancer biomarker c-Myc
• the inflammation marker NFκB
• the lung cancer marker KRAS associated with tissue rejection
• Botulism and Ricin
• Tocamycin, an antibiotic
• Uranium
• Mycoplasma pneumoniae

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http://BonhamLab.com

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