SensUs

SenseNC

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Summary for the SensUs website

The biosensor produced by SenseNC operates using disk-based assay technology. Small sample sizes containing NT-proBNP are loaded onto microfluidic channels on a disk and centrifugal force is used to move the sample through these channels. Detection antibodies specific to NT-proBNP and conjugated to quantum dots recognize and bind the NT-proBNP contained in the sample. Capture antibodies immobilized in the detection well bind the NT-proBNP-Detection antibody complex and immobilize the analyte for detection. The concentration of NT-proBNP is obtained using a UV lamp and photodiode to excite and detect, respectively, the quantum dots. The algorithm used by the machine enables rapid results from multiple samples.

1. Biosensor System and Assay

Bioconjugation:

The detector antibodies were regioselectively conjugated to CdS/ZnS core-shell type, amine functionalized quantum dots through oxidized sugar residues in the Fc region of the antibody in a process called reductive amination. The result of this conjugation is a stable, secondary amine bond between the detector antibody and the fluorophore. Capture antibodies are immobilized in microfluidic detection wells.

Assay:

Antibodies used in the assay were selected from those available based on a biolayer interferometry kinetics assay to determine affinity of each antibody for NT-proBNP. The biosensor uses Monoclonal mouse anti- human NT-proBNP antibodies provided by Hytest. The antibodies selected were 15C4 for capture and 29D12 for detector.

When a sample is loaded onto the sensor, the conjugated, detector antibodies bind to NT-proBNP. The antibody-quantum dot-analyte complex is bound by immobilized capture antibodies creating a sandwich. The quantum dots are excited by a UV lamp and emit at 450 nm. The potential difference of the fluorescence is captured as an image and linearly related to the concentration of analyte. The concentration of NT-proBNP is determined by signal transduction of fluorescence to voltage.

Microfluidics:

The assay disk is composed of PMMA. Microfluidic channels and chambers were laser cut from PET film and adhered to the PMMA layer. Four chambers are etched into the surface. The four chambers are linked by tear shaped channels to control stages of flow. Buffer reservoirs attached to the c

Capture antibodies are immobilized in the detection well. Multiple sample loading chambers and paths are etched into each disc, allowing for replicates of samples to be run simultaneously - leading to greater reliability and precision.

Mechanical:

A 24V, brushless DC motor is the main drive for the sample disc. This motor is controlled using a Maxon Motor control chip, and is capable of spinning a sample disc to a speed of 6170 RPM.

The sensor utilizes a CBT-39-UV LED with a 405 nm peak wavelength to illuminate and excite the quantum dots within each sample. This UV LED is capable of operation up to 21 watts, however the current sensor configuration is operating well below this threshold at approximately 0.7 watts. This is due to the sensitivity of the photodiode after optimization of the transimpedance amplifier, which required minimizing the effects of ambient UV light.

Two OPT301 photodiodes are used within the sensor to read samples from the detection chamber and control chamber. The photodiodes are placed behind a long pass filter that

effectively filters wavelengths from the UV LED, while allowing the color shifted wavelengths of light from the illuminated quantum dots to pass through. A high internal feedback resistance of 20 megohms was added to each of the photodiodes to increase the measurement sensitivity to adequate levels for quantum dot detection.

The sensor is controlled using a FRDM KL25Z microcontroller. This microcontroller is the brain of the sensor, which contains software to control motor speed, control the UV LED, and read voltage from each photodiode. Data is stored and processed on this control board, and results are shown via an LCD display or via serial connection to an attached computer terminal. A simple user interface is built in the sensor via external push buttons.

2. Analytical Performance

The transducer can detect quantum dots at the molar equivalent range of NT-proBNP for the competition standards.

3. Novelty and Creativity

3.1. Already available

We had access to analytical HPLC used for purification of conjugated antibodies. For selection of appropriate antibody pairs, access to the BLItz system by ForteBio assisted with the kinetic assay determination.

We had access to fabrication labs that assised with the disk creation, laser etching and other sensor design components 3D printers.

3.2. New developments

No one on our team had experience with antibody conjugation to quantum dots. With faculty advice from Dr. Menegatti and a good amount of research a procedure to conjugate amine functionalized quantum dots to oxidized sugars in the Fc region of the antibody was developed.

Signal transduction was accomplished with fluorescence instead of absorbance. Doing this required a means to filter out incoming light coupled with a power light capable of exciting the fluorophore. A photodiode with an on-chip transimpedance amplifier was chosen to minimize electronics footprint, and to increase the ratio of detection area to support circuitry.

The algorithm for reading the voltage signal output from the photodiode was developed entirely by the team. The methodology allows for multiple sample to be loaded onto the same disk and for discrete signals to be obtained for each sample by taking advantage of a robust but low power MCU.

4. Translation Potential

4.1. Healthcare application potential

Heart failure currently a prominent issue in the United States. In 2014, an estimated 6.5 million Americans were living with heart failure [1]. This number is expected to rise by up to 46% by 2030 [1]. The use of the SenseNC device could lead to better identification and monitoring of those with heart failure.

Currently, various imaging or blood tests are used for the diagnosis of heart failure including Magnetic Resonance Imaging, Electrocardiogram, or various blood tests [2]. However, these tests are costly, do not have a very high specificity, or take a long time to conduct. The SenseNC biosensor cartridges can be replaced, and eventually be made to run different samples at the same time, making this a very cost effective device. The combination of the high specificity of the test and short test time could potentially save lives, especially in cases of acute heart failure.

This device is expected to be used in diagnostic labs and point-of-care settings. The SenseNC biosensor would be suitable to be used in the emergency room to identify acute heart failure, or samples could be sent to a diagnostic lab where samples could be analyzed so a general practitioner could keep track of the progression of a patient's heart failure. The low operating cost of the device and its versatility will make it a valuable asset in numerous healthcare settings, regardless of whether it is being used for the diagnosis or management of heart failure.

4.2. Industrialization and commercialization potential

The SenseNC device will see changes in the future, as it becomes more cost effective, smaller, and faster. Currently, the estimated cost of each disc is around $10-$15. In the future, each disc will be able to handle multiple samples, and the cost of each disc will decrease as they would be mass produced. The overall size of the device will also decrease, possibly even to the point where the device could be handheld.

Currently, a competitor to the SenseNC biosensor does not exist in the American market, leaving it completely open to this device. However, before it is planned for industrialization, more time would be spent fine-tuning the device, including adjustments that will improve portability and versatility. These further developments would result a device that could be commercialized and economically viable. When these developments are actualized a device could be marketed with an array of analytes for full panel testing.

5. Team and Support

5.1. Contributions of the team members

Brendan performed kinetic determination of the antibody options with greatest affinity for NT-proBNP. Brendan led method development, performance and purification of antibody to quantum dot conjugation reaction. Additionally, Brendan did preliminary testing using lateral flow assays to assist with capture-detection pair determination of selected antibodies.

Bret assisted with preliminary design, and literature research on topics such as surface plasmon resonance, chromatographic methods, and ellipsometry. He also assisted in various lab procedures, including antibody to quantum dot conjugation, and antibody purification. Bret contributed to various deliverables, including slideshows and posters.

Calvin joined the team to contribute efforts to antibody purification and conjugation. Calvin instructed colleagues on laboratory practices including buffer preparation and spin filtration. Calvin also helped with the biotinylation of antibodies for the kinetics assay.

Alice initially contributed literature research in preliminary design. Alice assisted in producing written materials, such as presentations, buffer preparation, spin filtration, and quantum dot conjugation. Alice collaborated in the research and implementation of ellipsometry and surface plasmon resonance in identifying antibody binding properties.

Ben worked on designing and building the power system, motor drive system, and hardware controls for the device. He also assisted in creating the control and data analysis software.

Jack developed the CAD models, and fabricated the structural, housing, cable management, and thermal management components of the device.

Matt developed the control and display software for the device, as well as the data processing. He also assisted with circuit design.

Mike developed the optical transduction system and assisted in circuit design. He assisted in lateral flow assays, created thin film wicking material, and fabricated the microfluidics. He conducted device testing and characterization, managed team inventory and purchasing, assisted in graphic design, and helped coordinate international travel and shipping.

5.2. People who have given support

- Ashlyn Young: Lateral flow test and general advice for practicality of ideas.
- Dr. Stefano Menegatti: Conjugation methodology support and generous donation of lab materials and lab space.
- Dr. Daniele: Guidance and support too numerous to list in the scope of this document.
- Dr. Nathanial Hentz: Advised on assay development and generous donation of lab materials and lab space
- Ashton Lavoie: Generous donation of time in training on HPLC of team members and advice on filtration methods used during antibody conjugation.

5.3. Sponsors

- North Carolina State University: Provided funding for supply purchases, and provided lab space for research and sensor construction..
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6. Final remarks

This competition presented some unique challenges and provided ample opportunity to learn. Many members of the team were able to gain valuable experience in areas outside of their expertise that they may not have received otherwise.

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