Detection of B-Type Natriuretic Peptide with Flexible Biosensors for Machine Learning Based Cardiovascular Risk Assessment

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Introduction

Technology is becoming increasingly versatile as its size decreases. In particular, nanoscale biosensors offer new advantages for medical diagnosis and prognosis, including: *in vivo* implantation, real-time monitoring, and per-unit cost efficiency.

A biosensor was developed to detect elevated levels of B-type natriuretic peptide (BNP), a factor linked to heart failure. Devices were fabricated and characterized at the University of Pittsburgh in collaboration with the Signal Processing and Statistical Learning group and the Cardiovascular Institute of UPMC.

Process Overview

Fabrication	Testing	Analysis
Human Blood Serum Samples	PBS Baseline	Electrical Response
Biosensor + Surface Modification	Hemolysis Query	nD-PCA
	Human Serum + Mouse BNP	Neural Clustering
OLED Integration	Human Plasma	OLED Emission

Biosensor Fabrication

1. **Polyethylene Terephthalate** (PET) was chosen as the substrate. It has basic mechanical strength, chemical stability, and a flexibility that allows for a design versatility essential to biosensors.

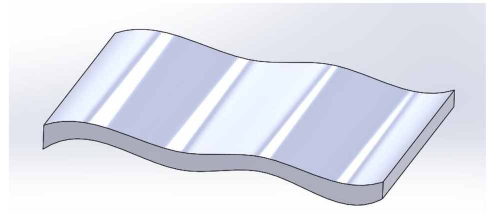

Figure (1): Flexible PET Substrate (translucent)

2. **Gold** (Au) was deposited onto the PET to form the electrodes. Gold has a high conductivity, low bio-toxicity, and resistance to oxidation and corrosion common to biological environments.

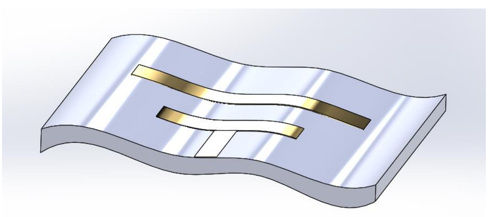

Figure (2): Gold deposited by electron beam

3. **Polyaniline** (PANI) was chemically synthesized onto the electrodes. PANI nanostructures exhibit excellent P-type semiconductor characteristics, and as film, PANI is compatible with flexible designs.

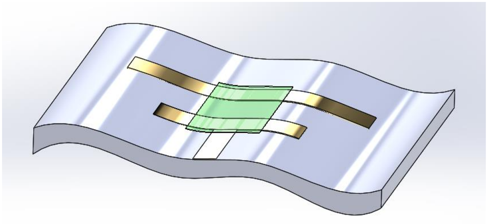

Figure (3): PANI thin film connects electrodes

4. **Surface-Modification** was performed to attach cardiac-factor antibodies to the PANI. A subsequent layer of BSA was added to increase specificity. Loaded antibodies serve as electrode bridge.

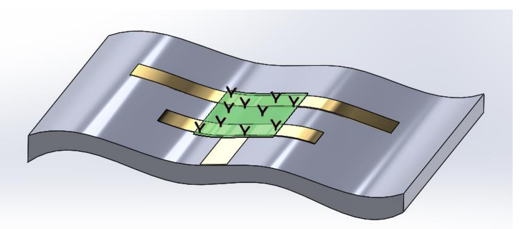

Figure (4): Antibodies create electrical line

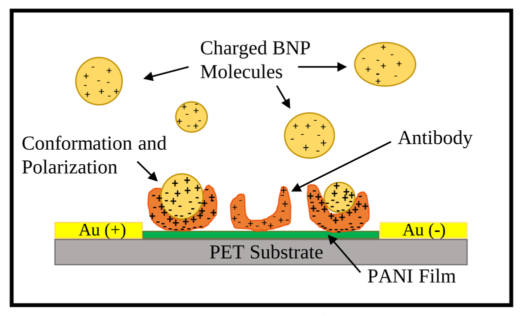

Figure (5): BNP-antibody complex forming electrode bridge

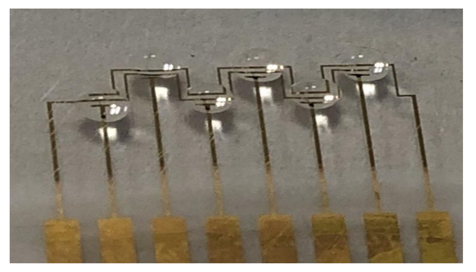

Figure (6): 6 devices testing and 6 sample serum droplets

Test 1: Specificity and Sensitivity

To test specificity, the devices were fed different biomarkers to confirm that BNP produced the largest signal. To test sensitivity, the devices were fed healthy human serum with varying BNP concentrations to find resolution of responsivity.

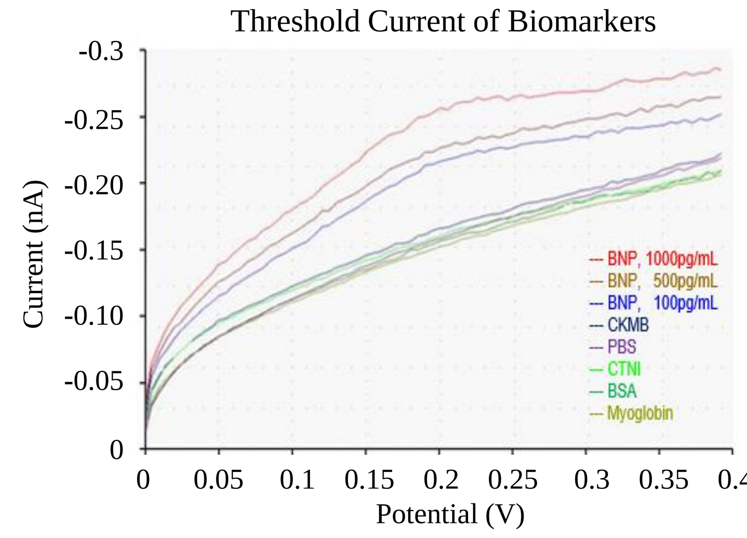

Figure (7): Current threshold for cardiac biomarkers

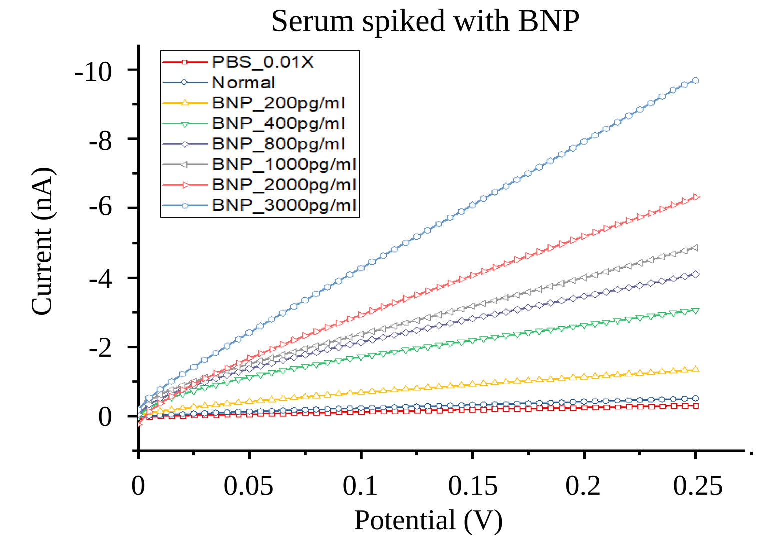

Figure (8): Current response to differing BNP levels

- BNP showed the highest current threshold out of various cardiac biomarkers
- The device successfully responded to samples according to concentration
- Significant signal differentiation began to occur at ~50mV

Test 2: Detection in Individuals

(N=10) Samples of healthy human serum were each measured on 10 separate devices to test for functionality with biotic samples.

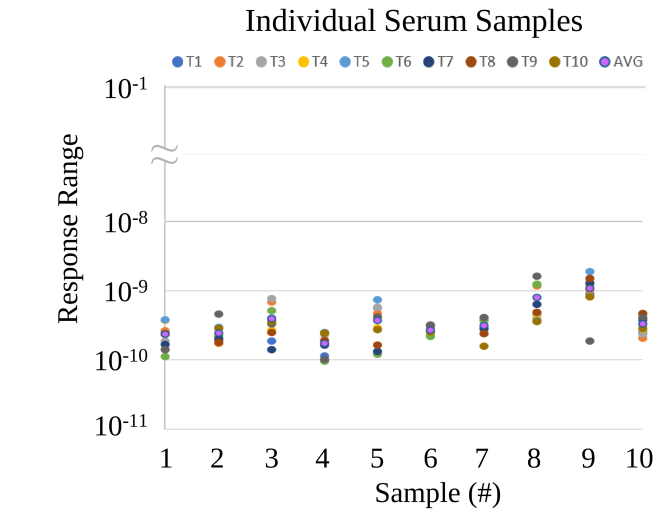

Figure (9): Conductivity of each sample

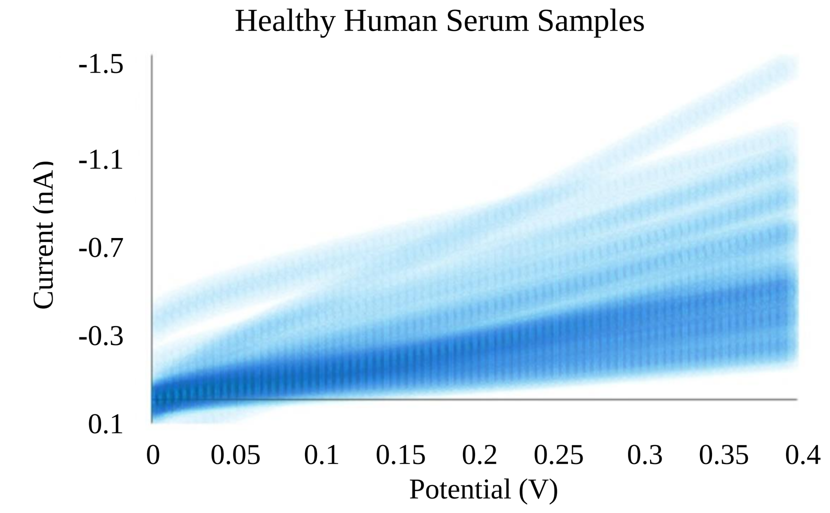

Figure (10): Overlay of sample voltage sweeps

- All PANI registered conductance, responses were confined to a tight range
- Baseline sensitivity of healthy BNP levels confirmed and established
- Signal linearity suggests chemical stability between PANI-receptors and BNP

Test 3: Blind Identification

A double blind test was conducted with a mixed group of 5 samples of varying concentrations (2 low BNP, 3 high BNP); each were tested on 10 devices.

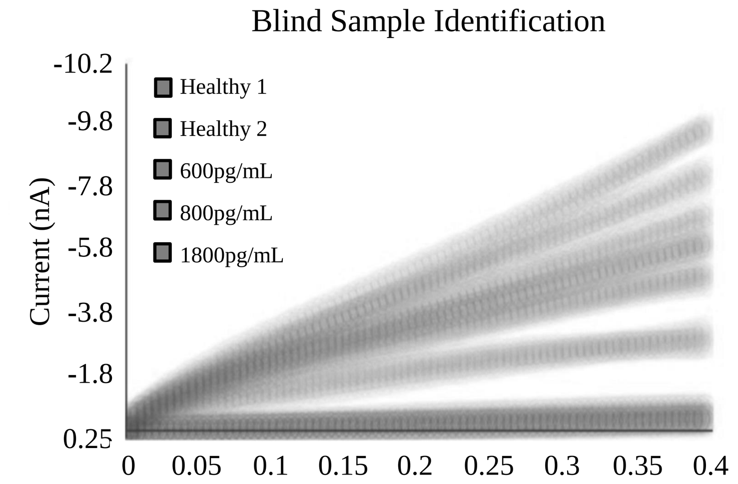

Figure (11): Measurements unidentified

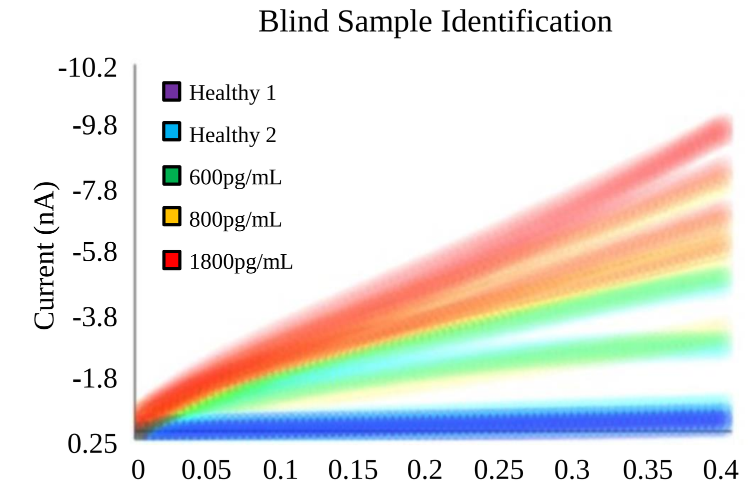

Figure (12): Measurements identified

- Slight variance within the higher concentration samples was observed
- Results were easily predicted from the unidentified data, showing consistency of sensor

Hemolysis Effect

Effect of hemolysis on conductivity were questioned. Red blood cells are non-polar, and hemolysis results in both insulating hemoglobin molecules and broken hemoglobin (ions), conducting. Sensitivity tests were conducted on different HGb:PBS ratios.

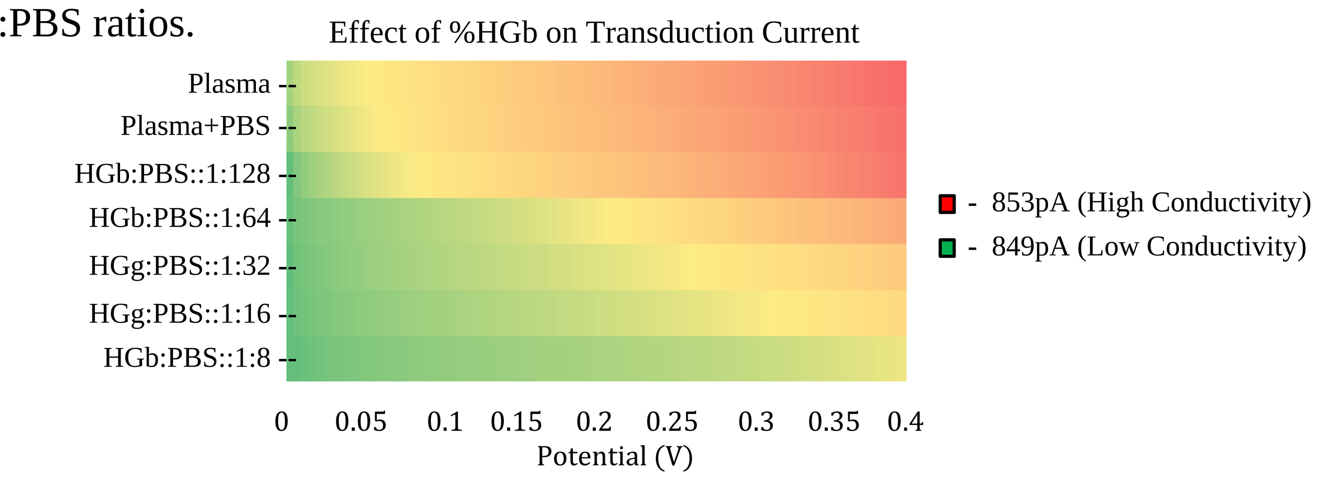

Figure (13): Overlay of sample voltage sweeps

- Compared to dilution, hemolysis in standard blood processing may be negligible
- Saturation thresholds occur in ranges HGb1:64-128PBS & HGb1:16-8PBS

Machine Learning

Classical statistics was also applied. 2-4 dimensional Principle Component Analysis (PCA) was used in conjunction with clustering to look at the data. Other factors such as patient race, sex, and age can increase dimensionality.

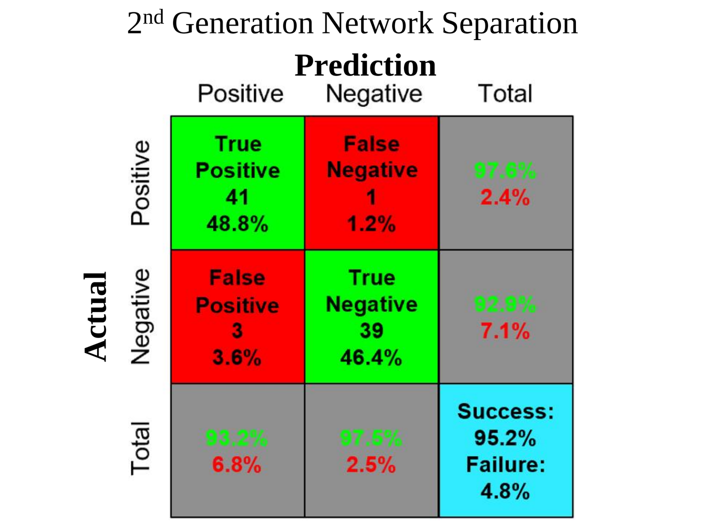

Figure (14): Separation of target data after 1 round of training

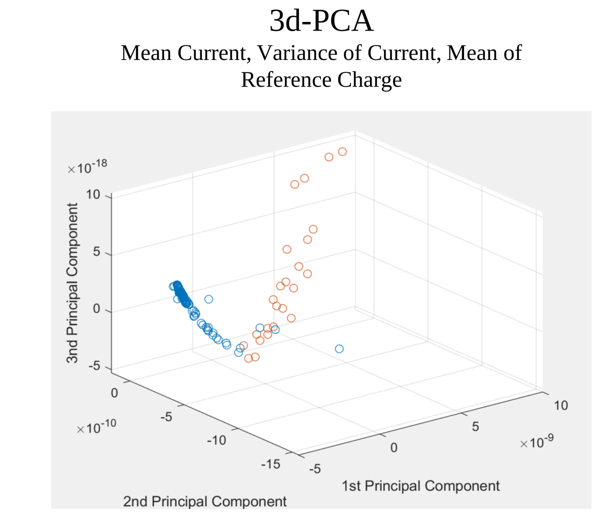

Figure (15): 3d-PCA, Mean(I), Var(I), Mean(Q)

- With more samples, the separator can be further trained to improve sorting
- In 3dPCA, 99/100 non targets and 28/30 targets were accurately identified

Conclusion & Future Work

The prototype biosensors were a success. A PET substrate allowed for flexible biosensors that were able to detect different BNP concentrations. Preliminary data was obtained from 15 human serum samples of different BNP concentrations and was characterized with PCA and neural clustering analysis.

This investigation allows for the separation of healthy and unhealthy BNP samples, which may helpful in identifying patients at risk of cardiovascular failure in the future.

Immediate work includes further delineation between healthy and at-risk targets, integration of OLEDs for visual indication, and training of a neural network.

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References

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