Intelligent Biosensor *(iBiosensC)* for Urine-Based Early Diagnosis of Breast Cancer

Sreyansh Mamidi[1], Deeptha Gokul[2], Remya Ampadi Ramachandran[3], Kai Yuan Cheng[2], Jing Su[4], Divya Bijukumar[2], and Mathew T Mathew[2,3]

[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA; [2]UIC College of Medicine, Rockford, IL, USA; [3]Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA; [4]Department of Chemistry, Northeastern Illinois University, Chicago, IL, USA

INTRODUCTION

- Breast cancer is the second most common cancer among women in the United States. In 2020, 2.26 million new cases of breast cancer were reported worldwide [1].
- Magnetic resonance imaging (MRI) and biopsies are **costly** and **painful** and only provide a diagnosis in the **later stages** of cancer.
- Electrochemical biosensors can be used for early cancer detection [2].
- Challenges: **(i)** the involvement of multiple biomarkers, **(ii)** big data analytics, and **(iii)** the accessibility of biosensors for a large population.
- **Urgent need** for an intelligent biosensor in breast cancer diagnostics *(iBiosensC)*, that uses machine learning (ML) and renders **early** results [3].

HYPOTHESIS & SPECIFIC AIMS

We hypothesize that **(1)** electrochemical biosensors can detect matrix metallopeptidase 9 (MMP-9), a breast cancer protein biomarker, in urine and **(2)** ML can make accurate predictions for early breast cancer diagnosis.

Aim 1: To **detect** MMP-9 in phosphate-buffered saline (PBS) and artificial urine through electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV)
Aim 2a: To **develop** a Python module to automate EIS raw data modeling and predict polarization resistance (R_p) and capacitance (C), **Aim 2b:** To **develop** an ML algorithm for *iBiosensC* breast cancer risk prediction

MATERIALS AND METHODS

Aim 1 – Materials

Material	Company
antiMMP-9 (200 µg/mL)	Santa Cruz Biotechnology
rhMMP-9 (500 µg/mL)	R & D Systems

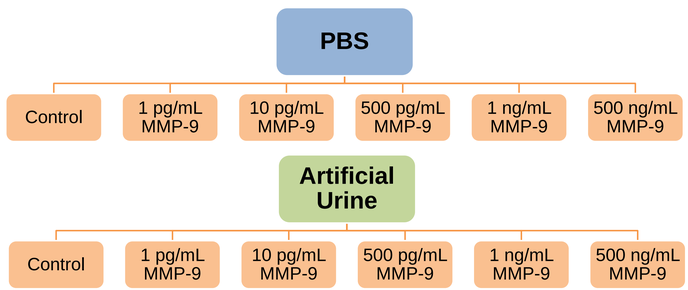
Material	Company
Screen-Printed Electrodes	Metrohm DropSens
Gamry Interface 1000 Potentiostat	Gamry Instruments

Composition of PBS:
Monopotassium phosphate – 0.144 g/L, sodium chloride – 9 g/L, disodium phosphate – 0.795 g/L

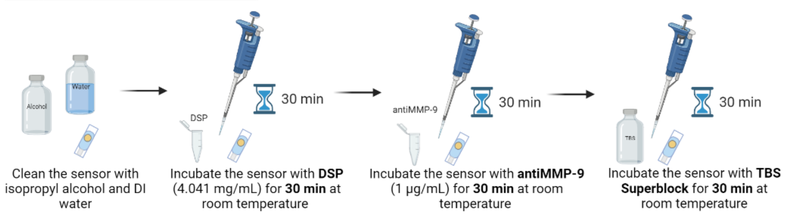
Composition of Artificial Urine:
Urea – 7 g/L, creatinine – 2 g/L, potassium chloride – 2 g/L, magnesium chloride – 0.5 g/L, calcium chloride – 0.25 g/L, sodium sulfate – 2 g/L, ammonium phosphate – 0.85 g/L, ammonium diphosphate – 0.85 g/L

Preparing Solutions of MMP-9

Constant working concentration of antibody: 1 µg/mL



Preparing the Biosensors



Clean the sensor with isopropyl alcohol and DI water

Incubate the sensor with **DSP** (4.041 mg/mL) for **30 min** at room temperature

Incubate the sensor with **antiMMP-9** (1 µg/mL) for **30 min** at room temperature

Incubate the sensor with **TBS Superblock** for **30 min** at room temperature

Gamry Electrochemical Tests

Open circuit potential (OCP) — 600 s → **EIS (vs. Eoc)** 0.01 – 10^4 Hz, **AC Voltage:** ± 10 mV → **CV (vs. Eref)** -1.5 – 1.5 Hz, **Max Current:** 25 mA → OCP — 100 s

Aim 2a – Methods

An **equivalent** electrical circuit with three elements – R_p, C, and solution resistance (R_{soln}) – was used to automate Gamry's EIS model. The following procedure was used to create the Python module.

Read Gamry Data → Set the Initial Conditions → Fit the Model → Extract the Fitted Parameters → Compare Gamry and the Module

Aim 2b – Methods

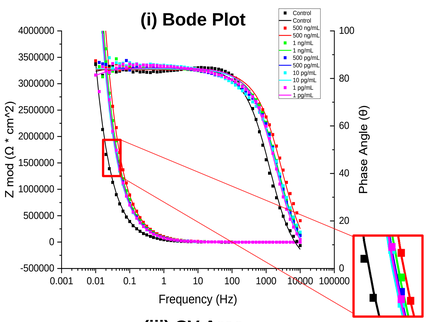
A support vector machine (SVM), or ML algorithm, was developed to **classify** EIS data into **three** levels: normal, low risk, and high risk. This was performed with respect to MMP-9 concentration.

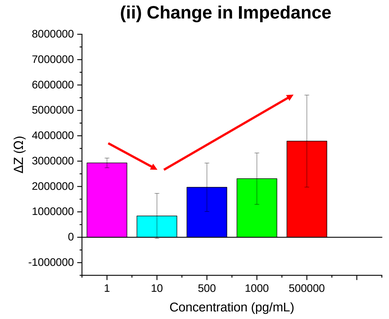
Level	MMP-9 (Urine)
Normal	0-1 ng/mL
Low Risk	1-20 ng/mL
High Risk	> 20 ng/mL

- **Four** features – change in impedance (ΔZ), R_p, C, and R_{soln} – were used to train the SVM.
- 80% of our dataset was used to train the model.
- 20% was used for validation.

RESULTS

A) MMP-9 Calibration in Artificial Urine





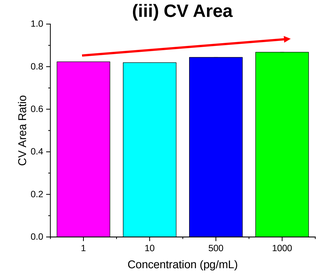


Figure 1:
(i) The control group has the lowest impedance (Z mod), and the 500 ng/mL group has the highest Z mod.
(ii) There is an initial decrease in ΔZ, as well as a clear increase for higher concentrations of MMP-9.
(iii) As MMP-9 concentration increases, a slight increase in CV area can be observed.

B) Statistical Test

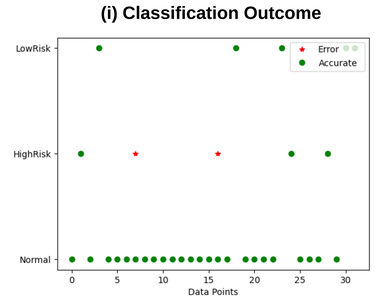
To compare Gamry and the Python module we developed, a two-tailed paired sample t-test was conducted using the experimental data from the artificial urine study.

***p* = 0.010** for R_p – **significant** difference in R_p ($p < 0.05$)
***p* = 0.448** for C – **no** significant difference in C ($p > 0.05$)

CONCLUSIONS

- The electrochemical biosensors can be used in **detecting the MMP-9 protein biomarker** in artificial urine. Our development of a Python module and SVM demonstrate that ML can provide **accurate predictions** of breast cancer risk.
- More *in vivo* studies that involve urine samples from breast cancer patients are needed to **establish** the **clinical significance** of an *iBiosensC*.

C) ML Risk Prediction – MMP-9 in PBS



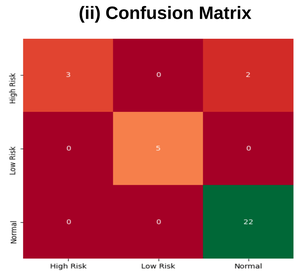
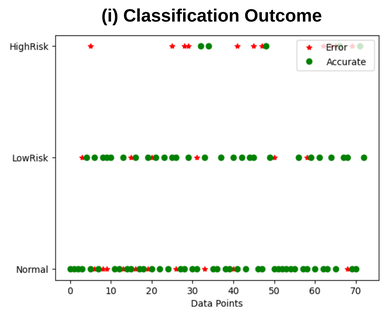


Figure 2: (i) and (ii) These figures demonstrate that for PBS data, the SVM had an accuracy of **94%**.

D) ML Risk Prediction – MMP-9 in Artificial Urine



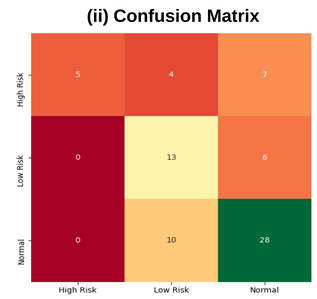
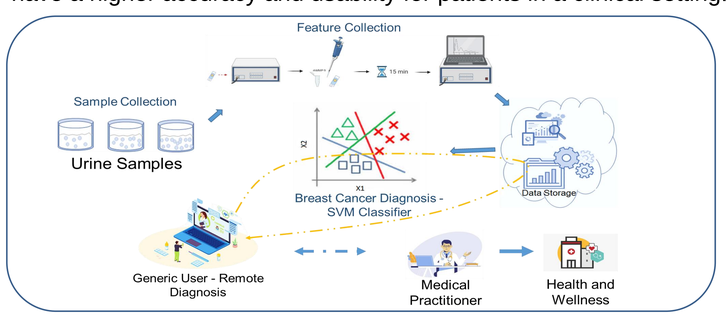


Figure 3: (i) and (ii) These figures show that the SVM had an accuracy of **63%** in the classification of artificial urine data.

DISCUSSION

- In this experimental study, we observed an **increase** in both ΔZ and CV area as MMP-9 concentration increased.
- Alharthi reported a **linear increase** in ΔZ with increasing concentration of ECM-1, another protein biomarker for breast cancer. [4]
- As polymers of amino acids, MMP-9's insulating nature likely contributes to a **decrease** in current flow and **increase** in impedance.
- The machine learning study was performed using a **limited dataset**. In fact, our experimental data was replicated to train the model.
- If the SVM was trained with **more data** and **less features**, it could have a higher accuracy and usability for patients in a clinical setting.



FUNDING

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[1] Cancer. (2021, March 03). Retrieved June 17, 2021, from https://www.who.int/news-room/fact-sheets/detail/cancer, **[2]** Alharthi, S.D.,et al *J Bio Tribo Corros* 7, 42 (2021). **[3]** Cui, F., Yue, Y., Zhang, Y., Zhang, Z., & Zhou, H. S. (2020). ACS Sensors, 5(11), 3346–3364. **[4]** Alharthi, S. D. (2018). [Unpublished master's thesis]. University of Illinois at Chicago.