Development of a novel Electrochemical Lateral Flow Assay for *in situ* biooanalyte quantification

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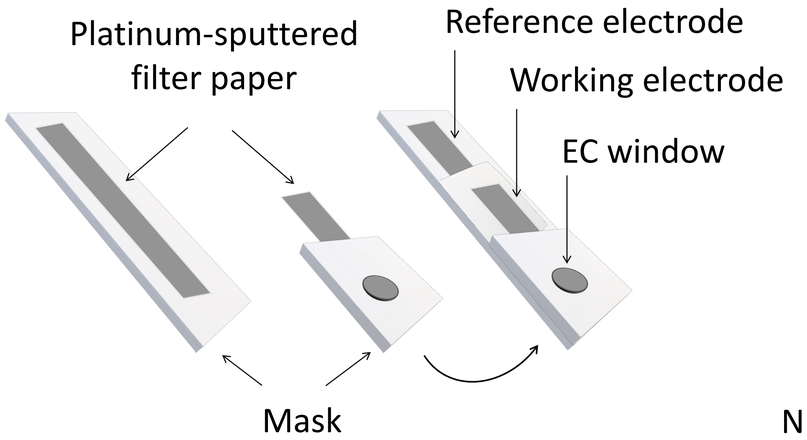
INTRODUCTION

The demand for quick and affordable obtention of biochemical information has increased over the years, but most tests only provide a qualitative response. To address this issue, we are developing a low-cost Electrochemical (EC) Lateral Flow Assay (LFA) device that quantitatively analyzes antigen-antibody interactions, using immunoglobulin G as a model analyte. The platform is made of platinum electrodes sensitive to hydrogen peroxide which is generated by glucose oxidase-labelled antibodies (GOX-Ab). By altering the mixed potential of the electrode, detection is accomplished. [1,2] The proposed methodology eliminates the need for additional enzymes and redox reactions, and the detection cell is made entirely of paper which allows easy integration into conventional LFAs with an added EC detection system. [3,4]

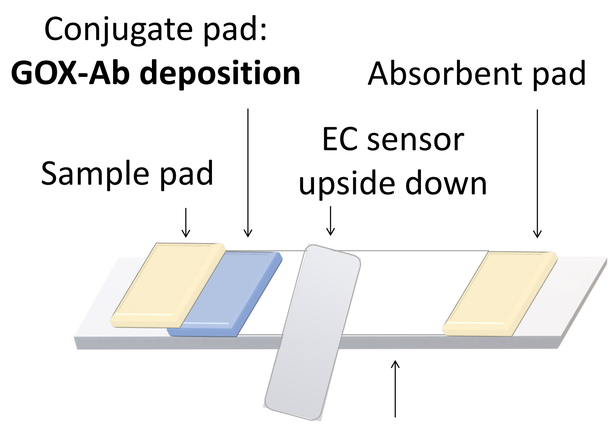
EXPERIMENTAL DESIGN

CONSTRUCTION OF SENSORS



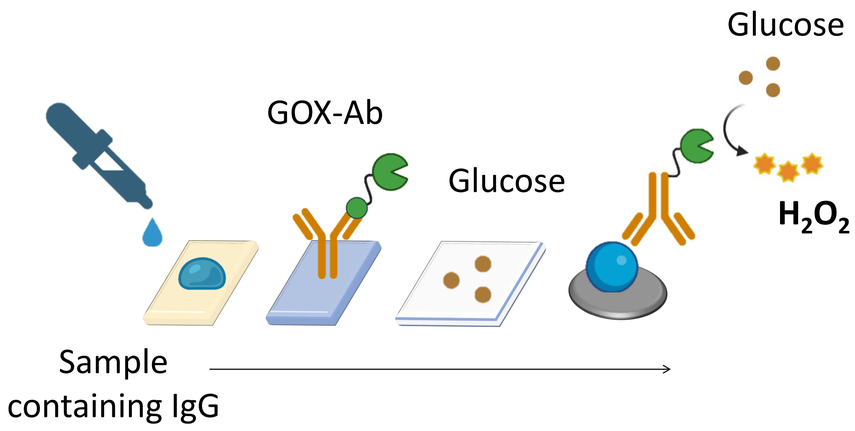
Nafion deposition on EC window:
10 µL on the back and 5 µL on the front

LFA PREPARATION



Nitrocellulose membrane: **glucose deposition**

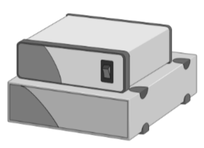
WORKING PRINCIPLE



D-Glucose + O_2 ⟶ D-Glucono-1,5-lactone + $\mathbf{H_2O_2}$

$\mathbf{H_2O_2}$ ⟶ $2H^+ + O_2 + \mathbf{2e^-}$

POTENTIOMETRIC MEASUREMENTS



Malvern high input impedance (1015 Ohm) multichannel EMF15 device (Lawson Laboratories, Inc. Malvern)

RESULTS AND DISCUSSION

EVALUATION OF PERFORMANCE OF SENSORS

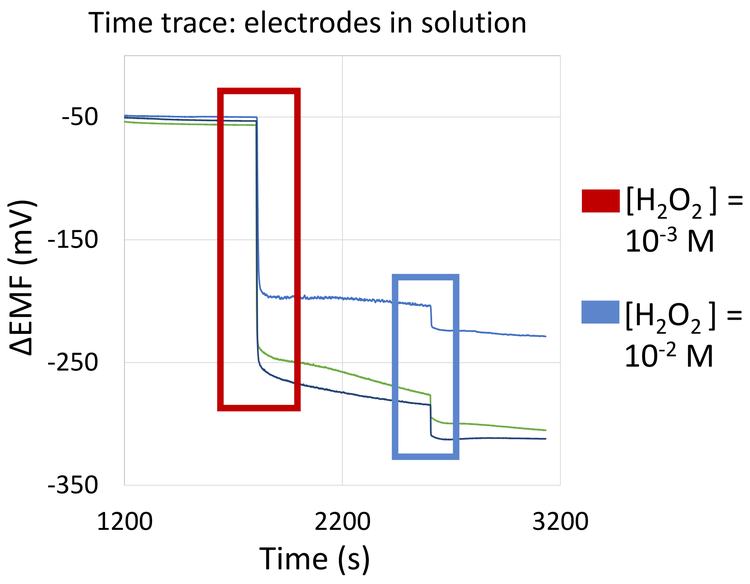


Figure 1. Three sensors (replicas) submerged in PBS 0,1M pH=7,4. Additions of H_2O_2 standard were made to accomplish concentrations of 10^{-3} M and 10^{-2} M.

PROOF OF CORRECT FUNCTIONING OF EC-LFA ASSEMBLY

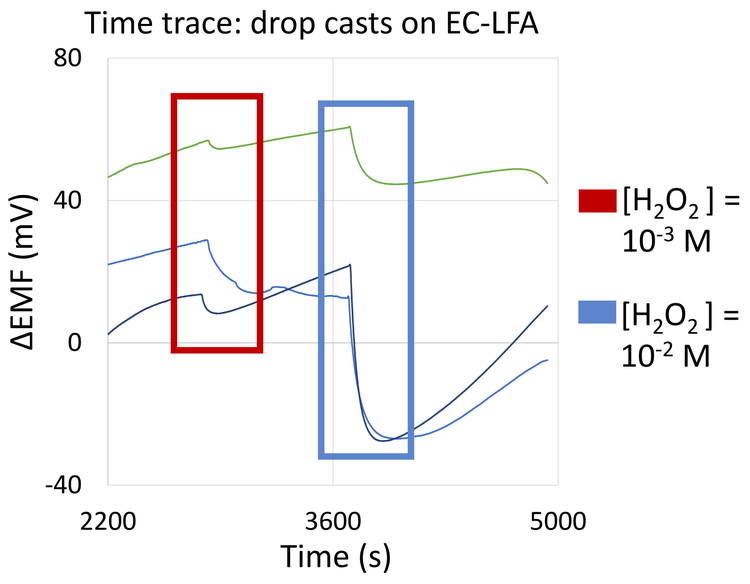


Figure 2. Three EC-LFA assemblies (replicas) were connected and drops of H_2O_2 were added: 10 µL of H_2O_2=10^{-3} M and 5 µL of H_2O_2=10^{-2} M

ASSESSMENT OF GOX LABELLING METHODOLOGY (*PROOF OF CONCEPT*)

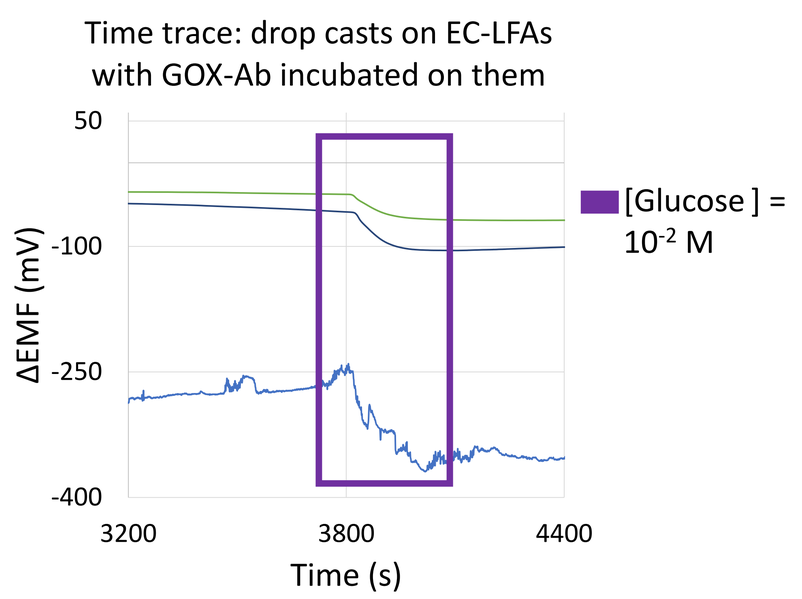


Figure 3. Three EC-LFA assemblies (replicas) with GOX-Ab previously incubated on conjugate pad. A drop of glucose was added: 10 µL of [Glucose]=10^{-2} M

CONCLUSIONS

- Sensors were proven highly sensitive to H_2O_2, providing a response of **ΔEMF > -150 mV for H_2O_2 of 10^{-3} M**, and **ΔEMF > -200 mV for 10^{-2} M**.
- Sensor-LFA **(EC-LFA) assemblies** resulted successful. Signal generated is proportional to the concentration of H_2O_2 added: **ΔEMF ≈ -10 mV** for additions of **10 µL of H_2O_2 of 10^{-3} M**, and **ΔEMF > -40 mV** for **10 µL of H2O2 of 10^{-2} M**.
- Proof of concept of GOX-label methodology has been demonstrated. Conjugate pads of EC-LFAs were previously incubated overnight with **GOX-Ab 20 mg/mL**. A mean response of **ΔEMF ≈ -50 mV** was obtained additions of **[Glucose] = 10^{-2} M.**
- Future work will consist of optimizing the stability of EC-LFAs to minimize disparity of results and ensure reproducibility.

REFERENCES

[1] Parrilla. et al. *Biosens. Bioelectron*. 2017, 90, 110.
[2] Andrade, et al. (2020). Potentiometric Hydrogen Peroxide Sensor (Patent No. EP20382133.5). Spain.
[3] Novell, et al. *Lab Chip*. 2014, 14, 1308.
[4] Perju, et al. *Anal. Bioanal. Chem*. 2021, 413, 5535.

ACKNOWLEDGEMENTS

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