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Colorimetric Detection of Cortisol Using an Alginate-Based Biosystem

Sepideh Izaddoust[1,2], Lourdes Basabe-Desmonts[2,3], Fernando Benito-Lopez[2],

[1]Microfluidics Cluster UPV/EHU, Analytical Microsystems & Materials for Lab-on-a-Chip Group, Analytical Chemistry Department, University of the Basque Country UPV/EHU, Leioa, Spain

[2]Microfluidics Cluster UPV/EHU, BIOMICs microfluidics Group, Lascaray Research Centre, University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain

[3]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

fernando.benito@ehu.eus

Summary:

This study highlights an alginate-based biosystem tailored for colorimetric cortisol detection, a vital biomarker in stress and health analysis. By integrating alginate beads with blue tetrazolium, the biosystem exhibits a visually detectable colour shift across cortisol concentrations ranging from 10 to 240 µg dL^{-1}. Calibration demonstrates 30 min as the optimal detection time with the highest accuracy. However, the system shows a slight decrease in accuracy for longer detection times, indicating partial instability over time. This innovation shows significant potential for clinical diagnostics and research applications in endocrine health.

Keywords: Lab-on-a-chip, Alginate hydrogel, Cortisol hormone, Colorimetric sensing assay, Microfluidics

Background, Motivation an Objective

Biocompatible alginate bead biosystems, incorporating enzymatic reactions, have been developed for rapids, colorimetric detection of glucose in sweat, achieving detection times of 13 min [1]. Alginate hydrogels are created by cross-linking linear polysaccharide chains, forming a 3D network that can retain a significant amount of water. Cross-linking can occur through physical and ionic methods using divalent cations like Ca^{2+} and Ba^{2+}. These methods enable the encapsulation of reagents within the alginate scaffold [2].

Cortisol, known as a stress biomarker, is a steroid hormone that partakes in many of the body processes such as regulating blood pressure, blood sugar and helping a correct immunological response are some of its main functions. In a healthy patient, the release of cortisol follows a well-defined circadian rhythm, modulated by the allostatic response to external stimulus. However, exposure to a chronic stress condition can lead to a higher-level of cortisol in the blood serum (> 25 µg dL^{-1}) which is known as Cushing syndrome and necessitate the early-stage detection of this syndrome.

To meet this purpose, we present an alginate-based scaffold that has been functionalized with specific chemical reagents to enable the rapid detection of cortisol. This system is meticulously designed to detect cortisol concentrations within the physiological and pathological (elevated) range found in blood by providing a reliable and efficient method for cortisol measurement, this technology holds significant promise for various applications in clinical diagnostics.

Description of the New Method or System

The colorimetric sensor was composed of alginate combined with blue tetrazolium (2.7 mM in methanol) and exposed to 1 M NaOH. Its functionality relied on the diffusion of components from within the alginate bead to the surrounding medium. To create the biosystem, alginate was dripped into a blue tetrazolium/calcium chloride solution (9:1) for 10 min. When NaOH and cortisol solutions were introduced, the blue tetrazolium diffused outward from the bead, reacting with cortisol in the NaOH environment. This reaction reduced blue tetrazolium to formazan, producing a colour change from transparent to purple. The colour intensity, measurable at 510 nm [3], directly corresponded to cortisol concentration (see Fig.1).

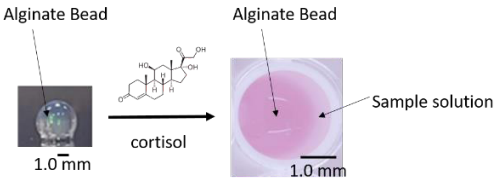

Fig. 1. Schematic representation of the performance of de alginate based biosensor.

Results

The colorimetric assay was fabricated by dripping 20 µL of alginate into a 9:1 solution of blue tetrazolium and 400 mM $CaCl_2$ using a syringe pump. Beads, left in the solution for 10 min, were spherical with an average diameter of 2.0 ± 0.3 mm. The obtained transparent alginate beads showed a pale-yellow colour demonstrating the entrapment of blue tetrazolium inside the beads. After rinsing with methanol and distilled water, the beads were transferred to a 96-well plate. NaOH (10 µL) and cortisol samples (150 µL) were added, and colour changes were recorded every 10 min for 90 min (see Fig.2). The evolution of the reaction between cortisol and blue tetrazolium over 90 min was observed for various cortisol concentrations (10-240 µg dL^{-1}). Results indicate a decreasing reaction rate over time due to the complete release of blue tetrazolium and the completion of the reaction.

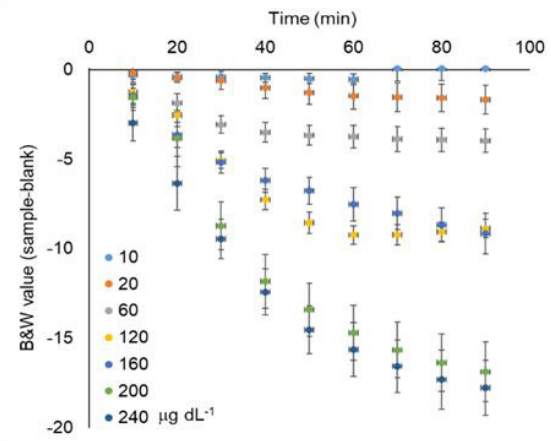

Fig. 2. Reaction of cortisol with blue tetrazolium using the alginate bead over 90 min for a range of concentrations 10-240 µg dL^{-1}. Error: (SD, n = 8).

The results demonstrated that a specific cortisol concentration produced a characteristic colour intensity at a defined time interval (see Fig.3).

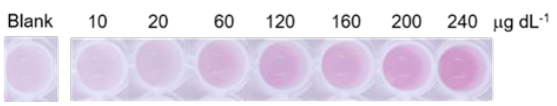

Fig. 3. Picture of the 96-well plate with alginate bead at different concentrations of cortisol, after 60 min reaction.

Calibration curves were constructed by subtracting blank values, enabling evaluation of biosystem efficiency for detecting cortisol across physiological and Cushing syndrome ranges. The optimal detection time was determined to be 30 min, yielding the highest accuracy. However, at longer detection times, a partial decrease in accuracy was observed, indicating potential biosystem instability (see Fig.4).

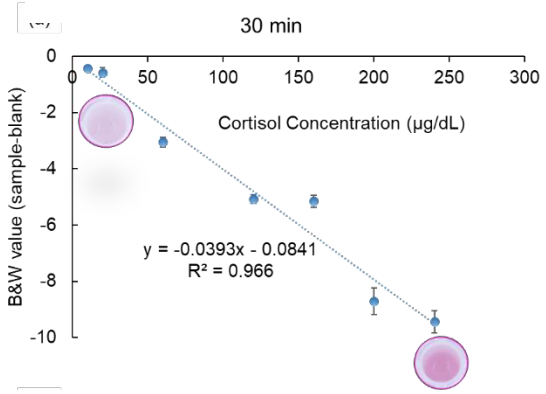

Fig. 4. Calibration curve for cortisol samples 10-240 µg dL^{-1} reacting with blue tetrazolium entrapped in alginate beads a) at 30 min. Error: (SD, n = 8).

Conclusions

This study explored a novel method for colorimetric cortisol detection. The feasibility of using blue tetrazolium as a chromophore for quantifying low concentrations of cortisol was assessed, and its integration into an alginate-based bead matrix was developed. In the near future, this biosystem can be used for the early detection and monitoring of endocrine disorders, as well as for assessing stress levels in patients.

Acknowledgements

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