Biosensors for Endogenous Lipids

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The endocannabinoid system comprises endogenous lipid mediators (endocannabinoids, eCBs) like *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), along with their specific G protein-coupled type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors, and the proteins responsible for eCB biosynthesis, inactivation, transport, and accumulation [1]. Growing evidence suggests that eCB levels might be modulated during pathological conditions affecting both central and peripheral nervous system [2]. The understanding of the biological significance of eCBs would not be possible without the development of methods for the accurate, precise, and sensible detection and quantification of these molecules in cells, tissues and biological fluids. Unfortunately, the currently available gold standard for eCB detection is liquid chromatography-mass spectrometry (LC-MS), a technique that, although highly sensitive, is expensive and rather sophisticated [3].

In the present study, a novel approach based on an innovative liquid biosensor has been used to analyse relevant eCBs: the BIONOTE device. The latter is composed of a probe and a dedicated electronic interface devoted to supply a variable input signal and to record the related output data [4]. The signal input consists of a triangular waveform between -1V and 1V and a frequency of 10 mHz while the output signal is the current response to a specific voltage input value. When the sensor probe is immersed in a solution, a current related to the oxy-reductive reactions occurring in the sample is recorded. Data is acquired with a sampling rate of 200 milliseconds, thus collecting 500 output values for each measuring cycle. The whole registered data array is treated as a global pattern with an enriched informative content representing the electrical signature of the analysed sample. The complex data set is finally analysed through multivariate data analysis techniques to provide a simplified representation of the multidimensional space acquired and to highlight the most informative features. Partial Least Square Discriminant Analysis (PLS-DA) coupled with the Leave-One-Out criterion as cross-validation method has been employed to obtain all the predictive models onto the calibration data. As a preliminary approach to eCB quantification, standards of the two most representative members of this lipid family have been analysed by the liquid sensor under controlled conditions. AEA and 2-AG were suspended in methanol at millimolar concentration and were measured through the BIONOTE, independently of each other. Despite the device was able to detect qualitatively both molecules, the micromolar error associated with the calibration curve was too large to allow detection of these eCBs at physiological levels. Due to the natural affinity to bind lipids circulating in blood, bovine serum albumin (BSA, fatty acid free) was selected as a candidate to attempt probe functionalization with the aim to improve the sensibility of the whole analytical system. A thin film of agarose gel containing 1 mg of BSA was then deposited by drop casting technique onto the surface of the probe, and then it was let to air dry. The modified probes were left to soak for 10 minutes in the target solution before starting the analysis. This process forced the dissolved lipids to move together with the solvent toward the dry BSA film where these got trapped by the binding with the serum protein. By means of this experimental setup we were able to increase the eCB concentration specifically at the probe interface. By challenging the BIONOTE with different amounts of eCBs to draw calibration curves in the 1 µM to 1 nM concentration range, and then applying a linear regression model to the experimental data, the system was able to predict both AEA and 2-AG concentrations with a Root Mean Square Error in Cross Validation (RMSECV) of 6.61 nM and 23.50 nM respectively. In addition, to test the discriminating performance of the analytical system against the eCBs, two molecules sharing part of the chemical structure of AEA and 2-AG were analysed: ethanolamine and glycerol. These substances were measured at the same concentrations as AEA and 2-AG, replicating the experimental setup. Also in this case, BIONOTE was able to predict the specific molecule concentration with an RMSECV of 4.47 nM and 20.73 nM, respectively. Finally, a comprehensive array containing the overall sensors' responses was built, and collected data were analysed through multivariate data analysis. The calculated PLS-DA models highlighted the ability of the system to distinguish the 4 standard molecules with an efficiency of 100% in the classification.

In conclusion, we report for the first time the development of an innovative lipid biosensor able to assess eCB content. Although our electronic device needs to be further validated by comparing its outcomes with those obtained by classical LC-MS analysis, the exploitation of sensors as suitable devices for high throughput screening of bioactive lipids has an apparent diagnostic potential for tomorrow's medicine.

[1] Maccarrone, M. *et al*. Nat Rev Neurosci 15 (2014) 786-801. [2] Di Marzo, V. Nat Rev Drug Discov. 7 (2008) 438-55. [3] Bisogno, T. *et al*. Eur J Lipid Sci Technol 111 (2009) 53–63. [4] Santonico, M. *et al.* Sensors 13 (2013) 16625-16640.

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Figure 1. Probe functionalization procedure.

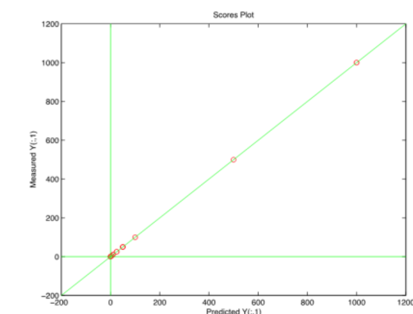

Figure 2. Predicted model obtained by a PLS-DA model. The device has been calibrated to AEA at concentration values ranging from 0 to 1 µM.

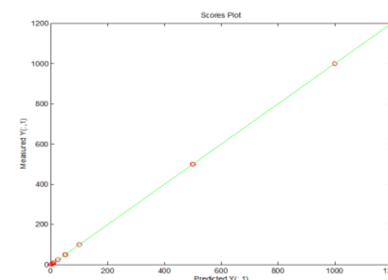

Figure 3. Predicted model obtained by a PLS-DA model. The device has been calibrated to 2-AG at concentration values ranging from 0 to 1 µM.