Carbon Nanotubes-Based Biosensors for Metabolite Monitoring in Cell Culture Medium

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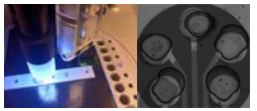
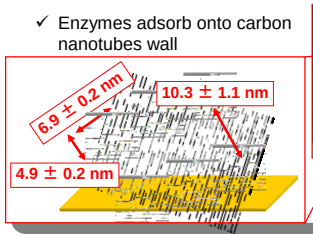
The poor knowledge about cell processes and differentiation mechanisms requires novel technologies to monitor the depletion of nutrients and the production of endogenous metabolites in cell culture medium. The present research aims to develop a self-contained platform of integrated amperometric biosensors to the real-time measurement of different metabolites over the cell culture duration. The immobilization of different oxidases onto carbon nanotubes (CNTs) confers high selectivity and sensitivity to the developed biosensor.

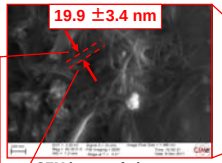
Some conventional techniques can be adapted to develop novel nanotechnology-based systems, leading to hybrid solution for new types of biosensors.

- ✓ Automatic spotting of CNTs is performed with a commercial non-contact spotter (sciFLEXARRAYER DW by Scienion). Spotter is typically used for DNA printing and microarrays. CNTs are diluted in Nafion, low aliphatic alcohols and water.
- ✓ Enzymes adsorb onto carbon nanotubes wall



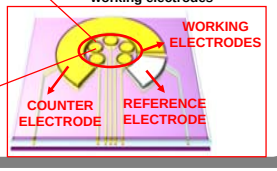
Non-contact spotting system for CNTs and enzyme deposition



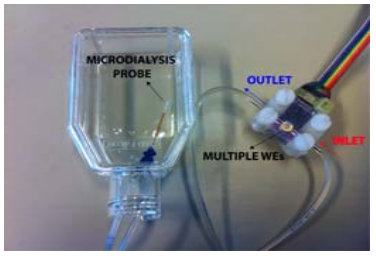


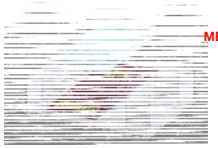
SEM image of glutamate oxidase adsorbed onto CNTs

Electrochemical cell with multi-working electrodes



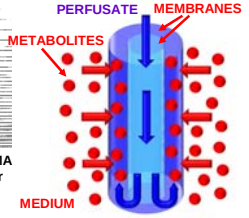
The fluidic system is used for two main purposes: to ensure a continuous recycle of fresh solution at the electrode surface, while products are removed; to dilute the culture medium, to perform proper electrochemical measurements.





In-house chip holder in PMMA hosting the PDMS chamber and the biochip

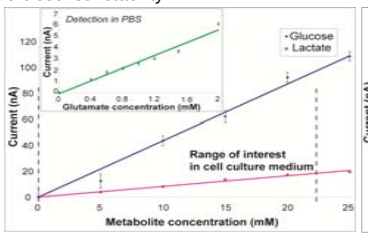
Working principles of the microdialysis probe



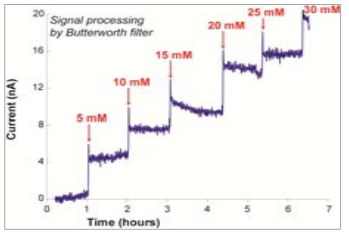
The microdialysis probe plays the role of a layer diffusion barrier. Such membranes with 6 kDa cut-off significantly extend the linear range of the biosensor, so that it is possible to cover the whole range of concentration of the investigated metabolites.

CNT-based biosensors are characterized in typical cell culture medium (DMEM/ glutamine + Foetal Bovine Serum) in continuous flow. Each working electrode is functionalized with a different oxidase to be sensitive to a specific metabolite.

Sensors are calibrated for the detection of three metabolites interesting to detect over the cell culture. Glucose and lactate detections are characterized in DMEM, while glutamate is measured in Phosphate Buffer Saline (PBS). The three metabolites are also detected over several hours to test the biosensor stability.



Calibration lines for glucose and lactate in cell medium. Inset: calibration line for glutamate in PBS solution.



Long-term measurement of lactate in cell culture medium.

Glucose and lactate biosensors show 10 times higher sensitivity compared to similar electrode modification reported in literature. On the other hand, concentration range for glutamate detection is much higher than what presented in other researches, even if the sensitivity is one order of magnitude lower than for the other reported biosensor.

	Technique	Sensitivity	Linear range	Limit of Detection
Glucose	MWCNT/Nafion + GOD [1]	4.7 µA/mM cm^{-2}	0.025 - 2 mM	4 µM
	MWCNT + GOD [2]	14.2 µA/mM cm^{-2}	0.05 – 13 mM	10 µM
	MWCNT/Nafion + GOD	55.5 µA/mM cm^{-2}	0 – 1 mM	2 µM
Lactate	MWCNT + sol-gel/LOD [3]	2.1 µA/mM cm^{-2}	0.3 – 1.5 mM	0.3 µM
	Au/Nafion/TNT + LOD [4]	0.24 µA/mM cm^{-2}	0.5 – 14 mM	200 µM
	MWCNT/Nafion + LOD	25.0 µA/mM cm^{-2}	0 – 1 mM	11 µM
Glutamate	PU/MWCNT + Glod/PP/Pt [5]	384 µA/mM cm^{-2}	0 – 0.14 mM	0.3 µM
	MWCNT/Nafion +Glod	0.9 µA/mM cm^{-2}	0 – 2 mM	78 µM

[1] Tsai et al. Langmuir 2005, 21, 3653 – 3658
[2] Wang et al. Electrochemistry Communications 2003, 5, 800 – 803
[3] Huang et al. Materials Science and Engineering C 2007, 27, 29 – 34
[4] Yang et al. Nanotechnology 2008, 19, 075502
[5] Ammam et al. Biosensors and Bioelectronics 2010, 25, 1597 - 1602

CONCLUSION

•We developed an integrated platform of amperometric biosensors for real-time metabolic monitoring of cell cultures.
•We immobilized different enzymes onto carbon nanotubes-modified electrodes.
•We calibrated the platform for independent detection of glucose, lactate and glutamate.
•We achieved to measure real-time metabolite variations in cell culture medium.

Research works

•Boero *et al.*, "Targeting of multiple metabolites in neural cells monitored by using protein-based carbon nanotubes", *Sensors and Actuators B* **2011**, 157 (1), 216 – 224
•Boero *et al.*, "Highly sensitive carbon nanotube-based sensing for lactate and glucose monitoring in cell culture", *IEEE Transactions on Nanobioscience* **2011**, 10 (1), 59 – 67
•Boero *et al.*, "New technologies for nanobiosensing and their applications to real-time monitoring", *in IEEE BioCAS Conference* **2011**, November, San Diego CA, USA

Neural cells from rat (SN56 cell line – magnitude 20X).