Amperometric biosensor with nanostructured electrodes by using multi-walled carbon nanotubes for glucose detection in cell culture medium

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The monitoring of metabolic compounds such as glucose is largely reported in literature. The applications of this type of analysis are mainly related to clinical purposes, e.g. in diabetes pathology, where a lot of studies are presented in literature. Recently, some authors presented studies about glucose and lactate detection in cell culture monitoring [1], [2]. A clear identification of medium compounds could be interesting for biologists and biotechnologists, since they may be identified as markers of different cell states. It can also pave the way to automated systems, as a feedback of the medium state.

In the field of amperometric biosensors, a lot of techniques related to the structuration of the electrodes have been presented in the last twenty years. Especially for glucose biosensors, a lot of mediators have been employed to carry the electrons released from the redox reaction to the surface of the electrode [3], [4]. Recently, some authors presented great results in terms of sensitivity and limit detection by using nanostructured electrodes. The employment of carbon nanotubes has shown promising results, due to their ability to promote the electron transfer from the active site of the enzyme onto the surface of the electrode, because of their electrocatalytic properties.

Since we observed an improvement in terms of sensitivity and detection limit by using Multi-walled Carbon Nanotubes (MWCNT) for *hydrogen peroxide* (H_2O_2) detection, we decided to modify the nanostructured electrodes with *glucose oxidase* (GOD), since glucose is the most interesting compound in cell culture. We dropped 40 µl (1 mg ml^{-1}) MWCNT onto Screen Printed Electrodes (SPE) purchased from Dropsens (Spain), and after, we deposited a certain quantity of GOD (3.5 U mm^{-2}) and we stored the electrode overnight at +4°C. The result of the detection from chronoamperometry in stirring conditions with PBS as support electrolyte is shown in Figure 1.

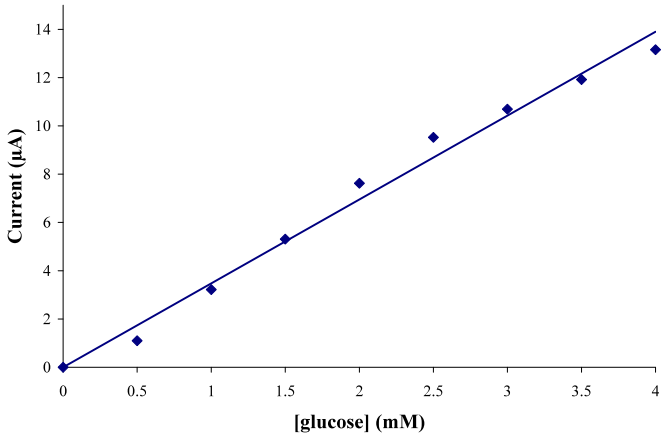

Figure 1 Calibration curve related to glucose detection with nanostructured electrodes by using MWCNT.

Our nanostructured electrodes for glucose detection show a sensitivity corresponding to 27.7 µA mM^{-1} cm^{-2} and a detection limit of 12 µM in a range from 0 to 4 mM. We obtained a value of sensitivity mostly higher than those reported in literature in the same range. Some better results were obtained by adding a polymer onto the surface of the electrode to entrap the CNT and the enzyme [5] or by functionalizing CNT with nanoparticles [6].

When the calibration curve was obtained, we carried out glucose detection in cell culture medium (Dulbecco's Modified Eagle's Medium from Sigma Prod. Code D5796). The cell line was type SN56 of neuronal cells from rat. We tested three flask with different cell density (FA = 5080 cell cm^2, FB = 25400 cell cm^2, FD = 203200 cell cm^2) and we detected the glucose amount.

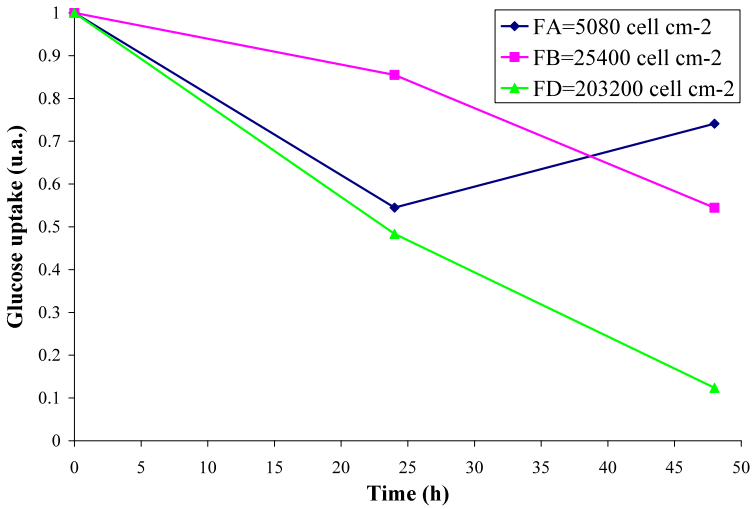

Figure 2 Glucose uptake in cell line SN56 after 24 and 48 h.

As it is shown in Figure 2, glucose uptake after 48 h from the seeding increases when cell density goes up. This result was what we were expecting, since it is practically well known that high density cell cultures need more often a refresh of the medium.

Future work will be focused in the design and optimization of amperometric biosensors for the detection of other metabolic compounds, such as hydrogen peroxide and lactate, to give us a more detailed description of the cell culture state. Final goal of the project will be the development of an array of electrodes for sensing these metabolites and integrating the required electronics for the detection in a chip.

References

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