# LACTATE AND GLUCOSE BIOSENSORS FOR SCANNING ELECTROCHEMICAL MICROSCOPY

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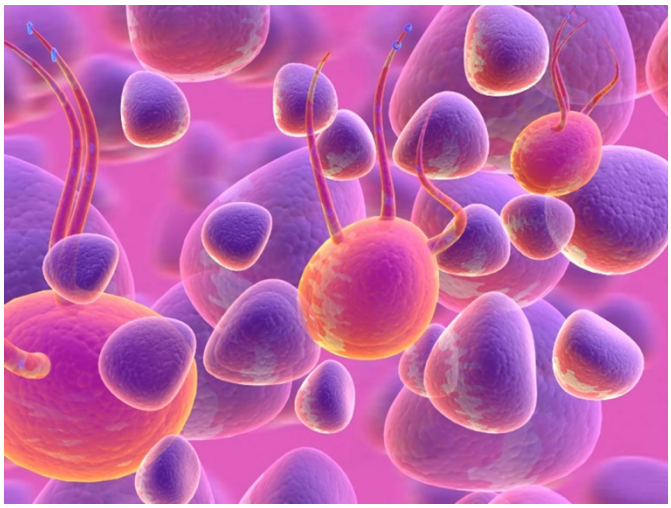

*In this work, a tool for the investigation of metabolic analytes, such as glucose and lactate is proposed. For this purpose enzyme-based ultramicroelectrodes (UMEs) are used in conjunction with Scanning Electrochemical Microscopy (SECM) to image metabolic species in the proximity of cells*

**Immagine elettrochimica dell'effetto Warburg su singola cellula**
Mediante lo sviluppo e l'utilizzo di biosensori enzimatici accoppiati alla microscopia a scansione elettrochimica (SECM) è possibile mappare due dei più importanti metaboliti coinvolti nel metabolismo cellulare (glucosio e lattato) al fine di studiare cellule tumorali.

Cancer is one of the most leading causes of death in the world; almost 9 million of deaths were counted in 2010[1]. Strategies to reduce at least exogenous risk factors are actively practiced or under investigation, nevertheless cancer incidence is increasing, also because of the population longevity increase. Therefore novel therapeutics and wide spreadable diagnostic approaches must be improved. In this view it is important to give a further insight in cancer metabolism and in cancer mechanisms in order to develop new routes for the prevention and diagnosis of this disease.

**Biological background**

In 1926, Otto Warburg reported that cancer cells are characterized by metabolic alterations of glycolysis[2,3]. Most cells use glucose as a fuel source. Glucose is metabolized by glycolysis in a multi step set of reactions resulting in the creation of pyruvate. In normal cells, much of this pyruvate enters the mitochondria where it is oxidized by the Krebs Cycle to generate adenosine triphosphate (ATP) to meet the cell's energy demands. However, in cancer cells or other highly proliferative cell types, much of the pyruvate is directed away from the mitochondria to create lactate through the action of the enzyme lactate dehydrogenase (LDH). In many normal cells, lactate production is typically restricted to anaerobic conditions (when the oxygen levels are low); on the contrary, some kind of cancer cells preferentially channel glucose towards lactate production even when oxygen is plentiful, a process termed "aerobic gycolysis" or *Warburg Effect* (Fig. 1)[4]. Indeed it was demonstrated that under aerobic conditions, cancer cells metabolize approximately tenfold more glucose to lactate in a given time than normal cells[5]. The reason of cell metabolism changes and of the glycolytic pathway switch occurring in cancer is still almost unclear, but in view of these fundamental discoveries, we are convinced that alterations to cellular metabolism should be considered a crucial hallmark of cancer and the investigation of these processes is mandatory to have a real contribution in the cancer field.

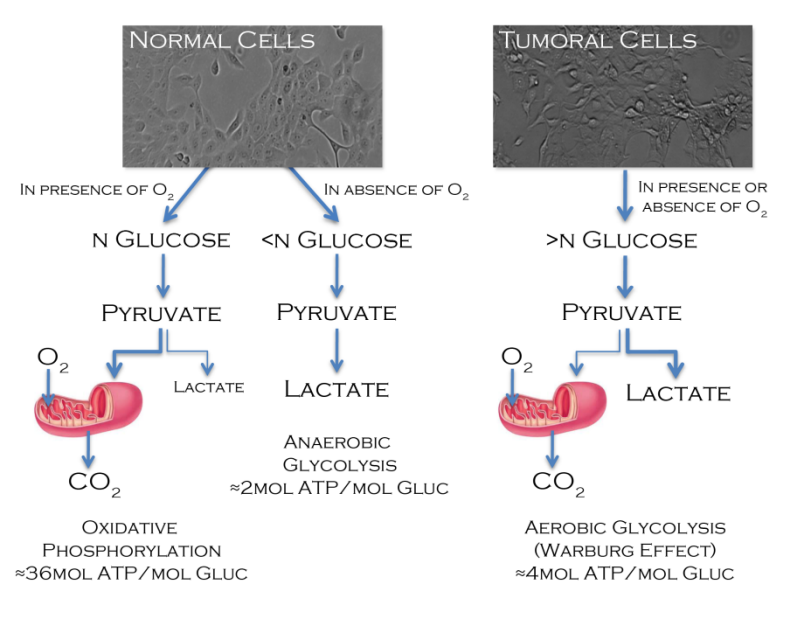


*Fig. 1*
*Simplistic way of metabolic pathways in normal and tumoral cells*

**Technology**

The past decade has seen a growth in the application of biosensors to micrometer and nanometer level investigations in a wide variety of disciplines. Rapid development, both in miniaturization techniques and in understanding of biological processes, has accelerated the expansion of biosensors in clinical applications and in areas such as biology, neurobiology, pharmacology and tissue engineering. Researchers have been able to acquire real time quantitative measurements through the application of microsensors on living cells, both *in vitro* and *in vivo*[6]. Of particular interest are the enzyme-based sensors because they offer high selectivity toward a single analyte, optimized by natural evolution, and they give the opportunity to improve sensitivity, time scale and information content. Enzymes achieve molecular recognition of substrates (i.e. analytes of interest) based on structural complementarity, leaving little space for error. They catalyze with high specificity chemical reactions and for this reason they have been successfully employed in miniaturization of sensor designs, including one of the most important sensor for the health industry that emerged decades ago: the glucose sensor, which is based on glucose oxidase (GOx) for *in vivo* monitoring of glucose levels or for diabetic's glucose meter. In addition to their importance for diabetic patients and in vivo studies, enzyme-based amperometric sensors are irreplaceable tools for the non-invasive study of the metabolism at the cellular level.

Enzyme-based ultramicroelectrodes (UMEs) in conjunction with Scanning Electrochemical Microscopy (SECM) can be developed as a useful technique for studying cell metabolic fluxes, since it can map electrochemical activity across the entire surface of a single cell with high spatial resolution and it can record dynamic changes.

Information about the way a cell performs glycolysis could be acquired using modified UME biosensors with glucose oxidase (GOx) and lactate oxidase (LOx)[6]. Glucose or lactate are measured indirectly by amperometric oxidation of hydrogen peroxide, that is formed in aqueous environment during the reaction catalyzed by the enzyme entrapped in the proximity of the electrode surface as described by the follow equations:

$$\text{D-glucose} + O_2 \xrightarrow{\text{GOx}} \text{D-gluconolactone} + H_2O_2$$

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOx}} \text{pyruvate} + H_2O_2$$

**Workflow and results**

We manufactured homemade GOx- and LOx-based biosensors, using several deposition techniques: including polymers and other entrapping systems[7,8,9]. Then by amperometric calibrations (Fig. 2) we tested the most important biosensors features: sensibility, stability, reproducibility, time of fabrication and spatial resolution, in order to find the best one.

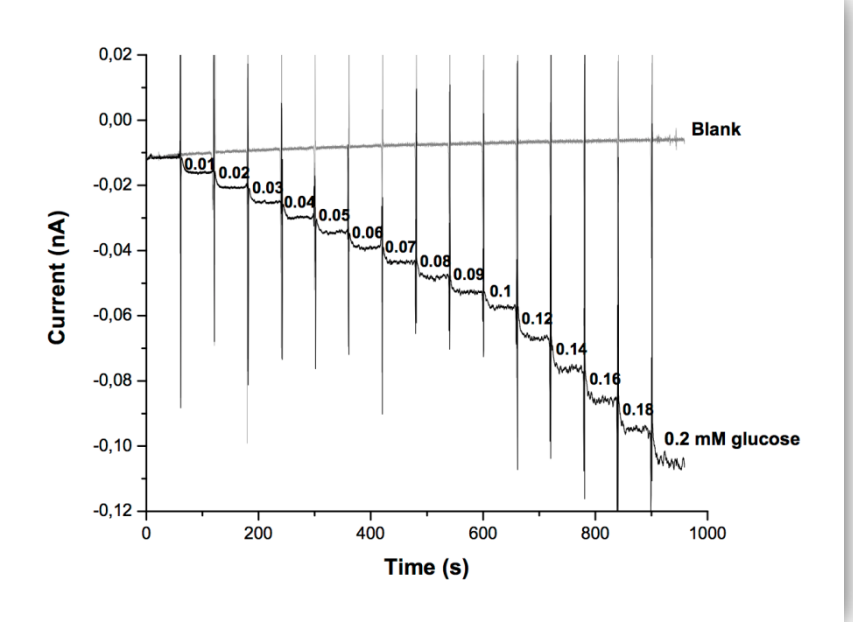


*Fig. 2*
*Amperometric calibration of dropcasted GOx 10 µm UME biosensor, E=+0.65 V vs Ag/AgCl, 3 M KCl; addition up to 0.2 mM of β-D-glucose (physiological range for single cell uptake) in saline phosphate buffer pH 7.4*

Now we are screening cells using these GOx- and LOx-UME biosensors. The test consists on amperometric signals recorded by the electrode approaching normal cells. Functional imaging of cell metabolism is possible scanning the electrode upon the cell surfaces. In the next future we would like to validate the method for testing the differences in glucose uptake and lactate release between tumor tissues and normal ones.

**Conclusions**

Using this powerful tool we are able to map both glucose uptake and lactate release at single cell level, and we foresee from this project the know-how to develop innovative diagnostic tools based on amperometric measurements of cancer metabolism.

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**REFERENCES**

[1]R. Lozano *et al.*, *The Lancet*, 2012, **380**, 2095.
[2]O. Warburg, The metabolism of tumors, Constable, London,1930.
[3]O. Warburg, *Science,* 1956, **132**, 309.
[4]S. Orrenius *et al.*, *Mol. Aspects Med*., 2010, **31**, 60.
[5]C.V. Dang *et al.*, *Nature Reviews Cancer,* 2011, **11**, 325.
[6]D.E. Cliffel *et al.*, *Anal. Chem.,* 2008, **80**, 2717.
[7]D.E. Cliffel *et al.*, *Anal. Chem.,* 2004, **76**, 519.
[8]D. Pan *et al.*, *Sensors and Actuators B,* 2005, **104**, 68.
[9]H-S. Kim *et al.*, *Biosensors & Bioelectronics*, 1996, **11**, 171.