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Photoluminescence Lifetime Based pH and O_2 Sensors for the Analysis of Cell Metabolism

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Summary:

Sensor based systems for cell analysis are well established and actively used in biomedical research and drug development. However, sensor materials and detection principles embedded in the current systems are not optimal. We describe advanced solid-state sensing materials which provide photoluminescence lifetime based sensing of pH and O_2 and calibration-free operation on existing detection platforms. They are demonstrated with mammalian cells and tissue samples, measuring their Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR).

Keywords: Photoluminescent oxygen and pH sensors; Luminescence lifetime based sensing; OCR and ECA measurement; Cell metabolism and bioenergetics;

Introduction

Optochemical sensors, mainly the photoluminescent O_2 and pH sensors, have been successful in cell analysis. By allowing high throughput contact-less measurement of the oxidative phosphorylation (OCR) and glycolytic (ECAR) fluxes of cultured cells under different physiological conditions, they enable a detailed analysis of cell bioenergetics and metabolism. The two main sensing approaches are Luxcel Mitoxpress probes and Seahorse XF (eXtracellular Flux) analyzer.

Luxcel's open architecture platform uses soluble phosphorescent O_2 and pH probes and detection on a standard plate reader in standard microplates (with oil seal for the OCR) [1]. Having moderate sensitivity, this platform provides highly accurate, multiplexed, calibration-free detection, via lifetime based sensing of both O_2 and pH. While the integrated opto-mechanical XF analyzer operates with dedicated microchamber plates reversibly sealed with moving pistons which also contain solid-state O_2 and pH sensor coatings. Although the XF allows user-friendly hands-free operation with sensitive and sequential OCR and ECAR measurement and effector additions [2], it uses non-optimal and outdated sensor chemistries and intensity based sensing of O_2 and pH. So, there is a need in improved sensing materials and detection systems for such applications, particularly for pH and dual pH/O_2 sensing.

Description of the New Method or System

Recently, we have developed a panel of new solid-state fluorescent pH sensitive materials comprising fluorescent porphyrin dyes embedded in plasticized PVC matrix, together with a proton transfer agent (a lipophilic borate salt) [3]. Such materials show prominent reversible response to pH (pKa 6-7, tunable), changing their fluorescence intensity, intensity ratio and lifetime characteristics. The ns lifetime based detection, which has internal referencing capabilities, is particularly useful, as it enables the design of robust calibration-free pH sensing systems. Furthermore, the new pH sensors are multiplexable with Pt-porphyrin based O_2 sensors which phosphoresce in the μs time range. The resulting dual pH/O_2 lifetime based sensors have the same detection settings: 380-410 nm for the excitation and 600-670 nm for emission. This simplifies their detection and temporal multiplexing.

Results

We have carried out rigorous characterization and optimization of the new lifetime based pH sensors, for use in pH/ECAR measurements. Optimization parameters included the type of pH sensing dye (OEP, OEPK) and PTA (several borate salts), their concentrations and concentration ratio in the sensor, pH calibrations (Abs and FI spectra, fluorescence lifetimes), determination of the corresponding pKa values and measurement ranges. Such sensor coatings were applied on planar substrates (Mylar film) and on 96-well plates, and reproducibility

of disposable calibration-free pH sensors and batch-to-batch variability were studied, so as possible toxic action of the sensors on cells, operational and storage stability, response time, cross-sensitivity with the O_2 sensors.

Thus, images of the OEPK based sensor dots in Figure 1 reveal that their Intensity signals are strongly influenced by dye concentration and sensor thickness, while lifetime signals are determined mainly by sample pH, producing narrow and distinct distributions at different pH.

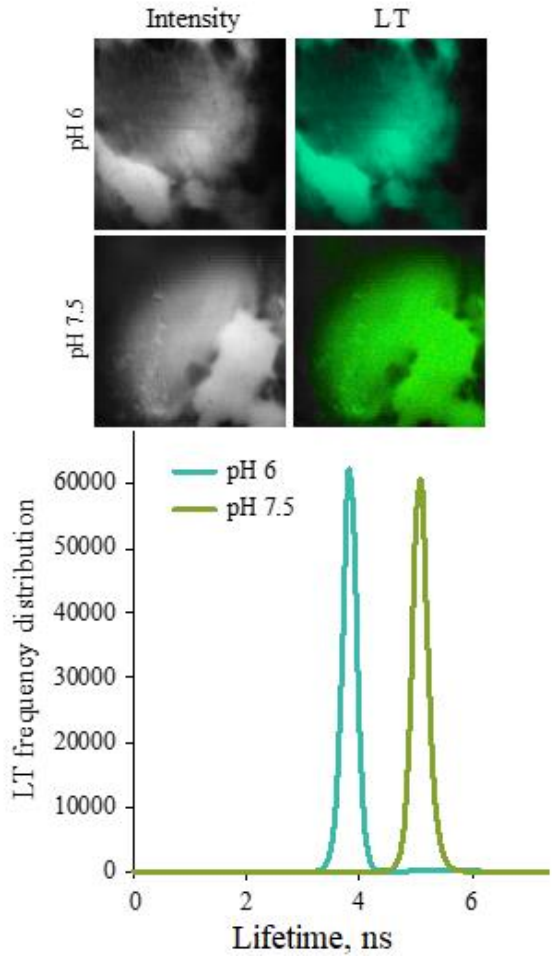

Figure 1. Fluorescence intensity and lifetime images of the OEPK based sensor coatings at pH 6.0 and 7.5 (top panel) and corresponding lifetime distributions (bottom panel), 21°C.

Since the sensors have stable and accurate LT calibration (Figure 2), they allow calibration-free operation on disposable or continuous basis for the measurement of pH and ECAR. Figure 2 also shows that the OEPK sensors are usable over the pH range 5.0 – 7.0 changing their lifetime from 3 ns to 6 ns, i.e. ~2-fold. And the OEP sensors work over the pH range 6.5 – 8.5 and have lifetime span from 7.5 ns to 12.5 ns.

Subsequently, the pH sensor coatings, deposited on suitable substrates (polyester film or polypropylene microplates), were demonstrated in the following biological experiments: i) ECAR measurements for HCT116 cells in 96WPs under different treatments [3]; ii) imaging extracellular pH in cultured multicellular spheroids formed by HCT116 cells [3], and iii) in ex-vivo tumor tissue and normal muscle tissue from mice [4]; iv) simultaneous ECAR/OCR measurement in HCT116 cells using OEPK/PtOEP dual pH/O_2 sensor coatings.

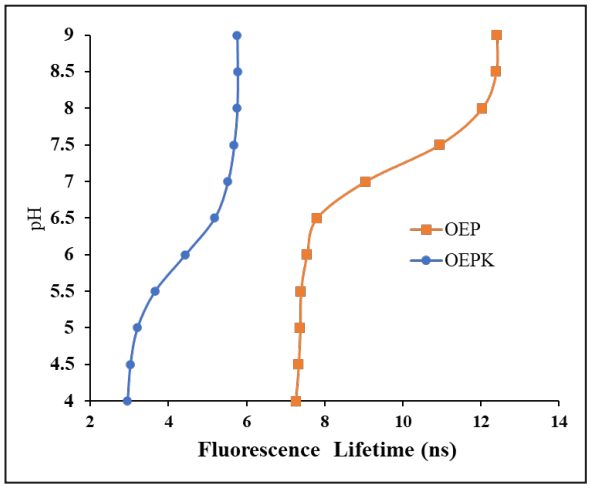

Fig. 2. Calibration curves for the pH sensors based on OEP and OEPK dyes in fluorescence lifetime scale, PBS, 21°C.

Overall, the new fluorescence lifetime based pH sensors and dual pH/O_2 sensors hold promise for use in cell analysis and tissue imaging.

Acknowledgement

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References

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