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High troughput, non-invasive and dynamic toxicity screening on adherent cells using respiratory measurements

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Abstract:

A dynamic respiration assay based on luminescence decay time detection of oxygen for high throughput toxicological assessment is presented. The method uses 24-well plates (OxoDishes) read with the help of a sensor dish reader placed in a humidified CO_2-incubator. Adherent primary rat hepatocytes and the human hepatic cell line Hep G2 were exposed to known toxic compounds. Dissolved oxygen concentration, a measure of respiration, was measured with an oxygen sensor optode immobilized in the centre of each well. The cells were maintained in the dishes during the assay period and can afterwards be processed for further analyses. This dynamic, non-invasive measurement allowed calculation of 50% lethal concentrations (LC_{50}) for any incubation time point giving concentration–time-dependent responses without further manipulation or removal of the cells from the incubator. Toxicokinetic profiles are compared with Sulforhodamine B assay, a common cytotoxicity assay. The novel assay is robust and flexible, very easy to carry out and provides continuous online respiration data reflecting dynamic toxicity responses. It can be adapted to any cell-based system and the calculated kinetics contributes to understanding of cell death mechanisms.

Key-words: Oxygen sensor, Respiration, Time resolved oxygen sensing, Luminescence decay time, Screening, Cytotoxicity