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Phosphorescent O_2 imaging probes for use in 3D tissue models

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The major importance of molecular oxygen (O_2) for living cells and multicellular organisms is reflected in its role in mitochondrial function, bioenergetics, metabolism, ROS production and signalling. Hypoxia and oxidative stress are implicated in many pathophysiological conditions including cancer, obesity, stroke and neurodegenerative disorders. O_2 tension regulates proliferation potential, senescence and viability of stem cells. Therefore, real time and dynamic monitoring of oxygenation in respiring samples such as developing neural cells is an important analytical task.

Phosphorescence quenching method allows for minimally invasive, quantitative analysis of O_2 in populations of cells by fluorescence spectroscopy on a plate reader or with sub-cellular level of detail by microscopy imaging. In recent years, a number of cell-penetrating O_2 probes have been developed which display different cell specificity and intracellular localisation. Their photophysical characteristics are compatible with different detection modalities: phosphorescence intensity, ratiometric or phosphorescence lifetime imaging microscopy (PLIM), under one- or two-photon excitation.

Here, we present application of nanoparticle- and small molecule Pt-porphyrin probes to O_2 imaging in 3D tissue models. Using neurospheres derived from primary embryonic rat brain cells, a common 3D model used for culturing and analysis of neural progenitor cells, we optimised staining of cells with the O_2 probes to achieve efficient in-depth accumulation and minimal toxicity. We then analysed oxygenation of respiring neurospheres, correlated their "hypoxic" cores with localisation of neural progenitor cells and with neurosphere size. In addition, we demonstrated multiplexing of O_2 imaging with analysis of neurospheres physiological status, their responses to hypoxia and stimulation with effectors such as glutamate.

Altogether, this methodology is useful for high-resolution mapping of cell and tissue O_2, assessment of metabolic, proliferation and differentiation status of neural progenitor cells in neurospheres and related 3D tissue models such as tumour spheroids and brain tissue explants of 100-500 μm size.

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