FAST 4D IMAGING OF ANTICANCER DRUGS UPTAKE IN MULTICELLULAR SPHEROIDS

Alessia Candeo[1], Abdullah R. Ahmed[1], Sofia D'Abrantes[1], Tord Kirkhus[2], Sarah Needham[1], Kristian K. Müller-Nedebock[3], Anthony W. Parker[1] and Stanley W. Botchway[1]
[1]Central Laser Facility, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Didcot, OX11 0QX, UK
[2]Department of Biological and Medical Sciences, Oxford Brookes University, Headington Campus, Oxford, OX3 0BP, UK
[3]Department of Physics, Stellenbosch University, Stellenbosch, 7600, South Africa
E-mail alessia.candeo@stfc.ac.uk
KEY WORDS: Multicellular spheroids, drug uptake, light sheet fluorescence microscopy

Multicellular spheroids (MCSs) are increasingly being used as tissue models by providing an advantage over two-dimensional cell monolayers by mimicking the physiology and functions of living tissues, making them suitable in tumour biology for morphology and drug screening applications. MCSs are currently used in cancer research, where cell signalling pathways can be elucidated. The drug AZD2014, a recognized inhibitor of the mammalian Target Of Rapamycin (mTOR) pathway [1], is undergoing active clinical trials, but its mechanism of action within a live cell environment is unknown.

Here we report the fluorescent properties of AZD2014 and we present the cellular uptake of the compound both in cell monolayers by confocal microscopy and MCSs by Light Sheet Fluorescence Microscopy (LSFM). This latter allows fast volumetric imaging of wide fields of view with cellular detail in a less phototoxic way compared to conventional microscopy. Moreover, LSFM has proved superior in detecting the fluorescence from MCSs compared to confocal microscopy [2]. The MCSs were imaged for up to 2 h after administration of AZD2014. The uptake rate was determined for different depths inside the MCS (Fig. 1) and showed a complex dynamic behaviour compared to monolayers, especially in the spheroid core, with possible interactions between the inner part and the surface. Volumetric changes difficult to observe in monolayers were also characterized, with an increase of the MCS radius of 20% upon drug administration and in presence of light (Fig. 2), suggesting a photo-activatable nature of the drug, confirmed also in monolayers. This work highlights the clinical significance of the combined use of LSFM and MCS models for the study of drug screening, of signalling pathways and photodynamic therapy.

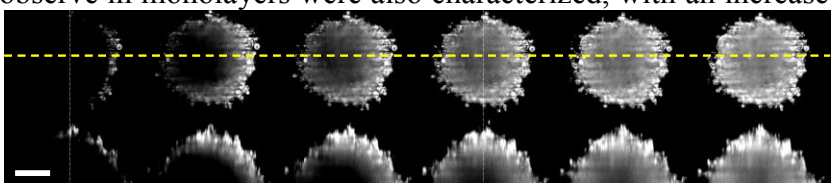

Figure 1: Uptake of AZD2017 from one MCS along 15 min post administration. The upper row shows a plane 120 µm in depth. The lower row shows the orthogonal projection of the line in yellow. Scale bar 100 µm.

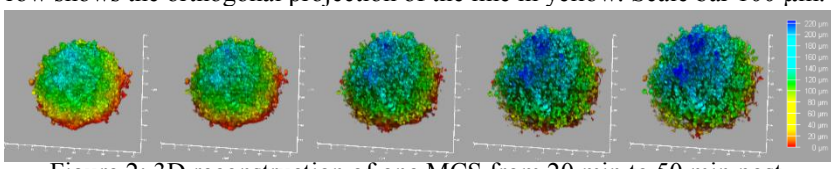

Figure 2: 3D reconstruction of one MCS from 20 min to 50 min post administration of AZD2014, showing an increase in volume with cell death. Colours are representative of different depth.

[1] K. G. Pike et al. "Optimization of potent and selective dual mTORC1 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014", *Bioorg Med Chem Lett* 2013.
[2] F. Pampaloni et al. "High-resolution deep imaging of live cellular spheroids with light-sheet-based fluorescence microscopy", *Cell Tissue Res* **352**, 161–177 (2013).